THE EMBRYOLOGY AND OÖGENESIS OF ANURIDA MARITIMA

(GUÉR)

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BY

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CONTENTS.

THE form chosen for this investigation is the small Collembolan, *Anurida maritima;* it is a form of very wide geographical distribution. It has been reported from many different places on the eastern and western shores of the Atlantic Ocean, and is probably present at many others. It received its specific name in Europe from the French naturalist Guérin in 1829-38. It has also been called *Achorutes maritimus* by Grube and *Lipura maritima* by Lubbock. According to the latest publications, Anurida belongs to the group Lipuridae, which, together with the Poduridae and Smynthuridae, form the suborder Collembola. Specimens were sent to Dr. H. T. Fernald, who confirmed their identification as *Anurida maritima.* The only contribution on the subject already published is one by Ryder in the *American Naturalist* for 1886, where

an adult, recently hatched young, and several stages of embryos are figured and described. All of Ryder's studies were made on whole specimens, and none of the early stages were figured.

The material was collected mostly during the summer of 1895 at Woods Holl, Mass., in connection with work done at the Marine Biological Laboratory. Both animals and eggs are found under stones, on sandy beaches, at about the level of half-tide, so that they are completely submerged for about half the time. They would thus be submerged to a maximum depth of about eighteen inches of water, the average tide being three feet in height.

Different methods of killing and preserving eggs and adults were used. The most satisfactory for the eggs proved to be the simple process of killing in hot water and hardening first in 70% and then passing to 95% alcohol. Owing to the thick membranes surrounding the ova, other processes failed for lack of penetration and resulted in shrinkage and distortion. Adults and young ones were killed in hot water and hardened in the same way as the ova ; those killed and hardened in warm and hot corrosive-acetic and picro-acetic acids and kept afterwards in 95% alcohol gave very good results. Owing to the difficulty experienced in wetting them, any method involving an early application of alcohol was easier than using water solutions entirely. Hot solutions were found better than warm, as they prevented contraction, causing the animals to die stretched out straight.

The material was almost all cut in hard paraffine and stained on the slide variously with borax carmine, Erlich's haematoxylin, lithium carmine and Lyon's blue, iron haematoxylin and orange G. Double staining was chiefly used and a few tests made with Biondi-Erlich. Sections were cleared in xylol and mounted as usual. In thickness they varied from five to fifteen micra.

Structure of Adult Ovary.

In specimens in which the eggs are nearly mature the structure of the ovaries is much obscured, as the abdomen appears to consist of a mass of eggs closely packed together and hence

irregular in shape. These eggs are not spherical nor even oval, but angular and compressed so as to entirely fill the abdominal and even part of the thoracic cavities. The number of eggs in a single individual varies from twelve to twenty; among the large mature eggs, which appear as masses of yolk, there are numerous small, immature ova, and in certain places masses of germinal epithelium.

Taking a stage where the eggs are less mature and yolk formation less advanced, the structure of the ovaries can be made out definitely. The organs are composed of a pair of simple tubes placed one on each side of the alimentary canal, as described by Fernald ('90). These are prolonged anteriorly into two long filaments, which reach into the second thoracic segment and finally coalesce with the fat body on each side at about the level of the middle of the alimentary canal in the vertical plane. Posteriorly the tubes retain their paired condition to the end of the fourth abdominal segment; at this point the tubes unite and form a single receptacle that may be called the uterus. This shortly passes into the oviduct, which is a median and unpaired opening at the hinder end of the fifth segment, and then directly to the ventral surface of the animal. Below this oviduct, as described by Fernald ('90), is a small sac opening to the outside by a common opening with the oviduct, which may be a receptaculum seminis; but careful search in many stages failed to reveal the presence of any spermatozoa.

Fig. 1, Pl. XX, is a longitudinal section through an ovary in which the eggs are just beginning to accumulate yolk; it shows the cephalic elongation, egg masses, and germinal epithelium. Nothing of the unpaired uterus and duct can be seen as the section passed to one side of these parts.

Four different structures are clearly shown. First, the cephalic elongation (Fig. 1, *c.el.*), which runs into a fine point and is attached laterally to the fat body lining the thorax. The structure of the thread is shown in Figs. 1, 5, and 6. It is a fine membrane in which nuclei are irregularly placed. These nuclei have no germinal characters; they are not to be distinguished from the nuclei of the wall of the ovary. This

elongation ceases in the middle or near the beginning of the first abdominal segment, and the ovary proper begins. This is in structure a simple tube, as shown in Fig. 1, in which are found many cells grouped round masses of protoplasm that differentiate clearly by staining in Lyon's blue. These cells are large and remarkably rich in chromatin of a peculiar arrangement. No nuclei are visible in the protoplasmic masses in the stages shown in Figs. 1 and 10, but in an earlier stage (Fig. 4), when yolk formation has not yet begun at all, a small, much-shrunken nucleus is distinctly visible. Caudad of these cell clusters lying in the third to the fourth abdominal segments is a large group of cells crowded together with indistinct outlines (Fig. 1, *g.e.*). This is easily recognizable as the germinal epithelium, and lies distinctly separated from the rest of the cells in the ovary. There is no definite arrangement of cells in this mass excepting a tendency to run in rows, which may lie in any direction, longitudinally, transversely, or dorsoventrally. The germinal mass is evidently non-metameric; there is no division of it into parts, and the whole is included within the third and fourth body segments. Numerous karyokinetic figures in the mass show that active proliferation is in progress. It is among the cells of some of these outer strings that the first differentiation is seen. Typically, the germinal cells are small and spherical; the cell bodies are very small and the nuclei large with the chromosomes regularly arranged on the periphery. Fig. 9 shows the first appearance of differentiation; in a line of cells among the typical round-nucleated cells appears one with a smaller, more oval nucleus containing smaller chromosomes which are still on the periphery. The development of a string of cells such as is shown in Fig. 9 into an egg mass can be followed in Figs. 2, 8, and 9. When the stage represented in Fig. 8 is reached the group of cells has passed to the outside edge of the germinal epithelial mass and is ready to fall freely into the ovary.

Returning for a more detailed consideration of the cell clusters, it is evident, as shown in Figs. 1, 2, 4, and 8, that there are many cells associated with that mass of protoplasm which contains, in early stages, the small, shrunken egg nucleus

(Fig. 4). This associated mass ultimately becomes the egg, the protoplasm containing the nucleus forming the definitive ovum and the surrounding cells bearing the relation of nutritive cells. Of the latter there are usually from five to eight grouped round one ovum; in the early stage they are not distinguishable from the general mass, but at a certain time an increase in size and also in the proportionate amount of chromatin is marked. This, together with the simultaneous decrease in size and change in shape and in amount of chromatin of the ovum nucleus, serves to emphasize the suddenness of differentiation. Fig. 10 shows the characteristic position of the ovum surrounded by a semicircle of nutritive cells. The change in the appearance of the chromatin can readily be seen by comparing Figs. 2, 9, and 10. In Figs. 2 and 9, the youngest stages, the chromosomes are rather small, irregular masses on the periphery, usually eight in number, with a more centrally placed nucleolus. In the later stage (Fig. 10) the chromosomes have assumed a stellate structure of a most pronounced type; they are usually eight in number with a more central and non-stellate nucleolus. In the same figure there is an additional change. There appears in the cell body close to the nucleus a mass of material clearly of a distinct chemical nature. It forms a cap, as it were, on one side of the nucleus, usually the side next to the ovum; by its accumulation it pushes the nucleus out of its hitherto central into an eccentric position. The presence of this material in the nutritive cells indicates a young stage in the ovum; yolk has not yet appeared in any large quantities.

One more point must be noticed in this connection. There is always associated with the nutritive cells, usually one on each end of the string, a cell of distinctly different character. This is shown at *a.o.* in Figs. 10 and 11. It entirely lacks the characters of the nutritive cell. It is smaller, has an extremely small cell body and large nucleus with eight minute chromosomes, which are at first arranged on the periphery, as is the case with the nutritive cell. There are one or two large nucleoli easily distinguishable from the eight chromosomes. Later the chromosomes move inward and arrange themselves in groups of four (Fig. 13). These cells have without doubt

some of the characteristics of the true ovum, and are ova that have passed in the course of development up to a certain point. The nuclei must then have undergone a degenerative hypertrophy, as they are much larger than those of the developing ovum of the corresponding stage. The cell body grows smaller and takes the Lyon's blue stain strongly on double staining after lithium carmine. The ultimate fate of these cells is the same as that of the nutritive cells, — degeneration and absorption.

The last stage in which the nucleus of the ovum is recognizable is shown in Fig. 4. The egg now consists of an irregular vesicular mass of protoplasm which forms the cell body in which a very small nucleus or germinal vesicle is eccentrically placed. This nucleus is irregular in outline with very small, peripherally arranged chromosomes, the number of which it was impossible to determine exactly; by inference it would be eight. The germinal vesicle has every appearance of losing its nuclear membrane; if this is the case, its apparent disappearance after this stage is attributable to the difficulty of distinguishing the separated minute chromosomes. In confirmation of this point a few observations were made on another apterygote, a thysanuran (*Tomoceras sp.?*), in which eggs were found in a similar stage as well as some a little more advanced. The cells are much larger in these animals and the ova are distinctly large masses of vesicular protoplasm with a small group of eight chromosomes eccentrically placed, not surrounded by any nuclear membrane (Fig. 14, *g.v.*).

In the next stage in Anurida, as shown in Fig. 10, the nucleus is no longer visible, the protoplasm has increased in quantity, and has also become more vesicular. In some of the spaces of the network yolk has already begun to appear, as is shown by the deep staining of parts in Lyon's blue. The nutritive cells have grown in size and the stellate structure of the chromatin is clearly defined. That these stellate masses are the chromosomes is shown by the use of the Biondi-Erlich stain. In this the stellate masses appear dark green, almost black at the central, more dense spot, and lighter green in the radiating strands; the non-stellate masses take the bright red

of nucleoli. These masses vary in number from one to two. The cell bodies of the nutritive cells have increased greatly in size, and at this stage show the differentiation into two kinds of material most clearly. By the use of lithium carmine and Lyon's blue the contrast is rendered most vivid. The chromosomes and nucleoli stain a brilliant red in the carmine, differentiating the nucleolus as a peculiarly refractive body. The cell body protoplasm stains a rather purplish red, being affected to a slight degree by both stains. The cap of different material comes out a brilliant blue, as also does the yolk present in the protoplasmic meshes of the ovum. Although no membrane can be found delimiting this blue material, its edge is as clear cut as if such a condition existed. From the constantly circular appearance of the nuclei of the nutritive cells when cut in all planes it is evident that they are spherical, and hence the blue material must cover the nuclei as a cap on the side facing the egg, thus causing the nucleus to take an eccentric position.

While these changes are taking place in the ova and nutritive cells the wall of the ovary begins to grow round each of the cell masses, and finally the ovum and the cells that contribute to its formation are inclosed together in a more or less completely separate sac (Fig. 4, *f.*). During the period of yolk formation the follicle is gradually stretched more and more, and hence becomes thinner and thinner. There is no evidence that the follicular cells divide either kinetically or akinetically, as has been described by Wheeler ('89) in Doryphora and Blatta. This thinning process is continued until, as shown in Figs. 11 and 12, the follicle is hardly distinguishable. At no time is it a prominent feature of the ovary, and it becomes more and more insignificant from the beginning of yolk formation. Its slight development is a most potent argument against its taking any active part in yolk formation. Commonly the follicular cells either completely or in part supply the elements of this process. In many cases, as shown by Stuhlmann ('86) and others, a highly developed follicle is present; also an ovarian wall and nutritive cells. As a rule, however, the degrees of the development of the follicle and the nutritive cells stand in inverse ratios, the work of aiding the egg in

yolk secretion being performed by either one or the other. In the case of Anurida the inference is that the nutritive cells take an active part in the yolk formation. Their great growth during the early stages of the egg, the presence of differentiated material in them, its disappearance, and, following this, the consequent appearance of yolk in the egg, the continued vigor of these cells until the full size of the egg is attained, and then their gradual absorption, are all facts pointing to the functional activity of the nutritive cells and their direct relation to yolk formation.

Fig. 11 shows the nutritive cells at their stage of greatest development. The increase in the size of the eggs and the consequent crowding in the body cavity have together given the nutritive cells angular instead of rounded outlines. It is, moreover, hard to determine always to which egg they belong. There is no increase in their number from the beginning.

The next step is the formation of the egg membrane and the degeneration of the nutritive cells. From the very slight development of the follicular cells it might be inferred that they take no part in the formation of the envelope of the egg. This is found to be true, and the process of formation by the egg itself is as follows: When the full size is reached the outer surface of the yolk undergoes a process of disintegration. Large yolk granules are broken up into smaller ones, and these in turn are finally transformed into a thin protoplasmic layer which shows clearly its network structure. This mesh-work gradually becomes obliterated, first on the outside and later farther in, as it were by condensation, until it shows an almost homogeneous structure outside preserving its fibrillar character inside (Fig. 12, *e.m.*). From the beginning, it stains deeply with Lyon's blue, a fact true of the envelope of the egg in later stages. Its formation is almost simultaneous over the whole surface, excepting in the region of the nutritive cells, where it appears a little later, a fact observed by Korschelt ('89a) in other insects. Fig. 12, *e.m.*, shows the envelope in an advanced state. The hardened homogeneous exterior already exists and the nutritive cells are separated from the ovum. They have shrunk in size; the cytoplasm has almost entirely

disappeared; the chromosomes have lost their stellate structure and are fused into irregular masses. The whole cell appears as a mass of deeply stained chromatin. The final stage when the egg envelope is fully formed and the nutritive cells have entirely disappeared is reached before the egg is laid, no sign of any outside cells being present in the last stages of the ovarian egg.

It is clear from the foregoing description that the envelope formed is not a chorion in the true sense of the word. It is a "Dottermembran," "Dotterhaut," such as is found among crustaceans, and is described in many myriapods. Schmidt ('95) in describing Pauropus designates the egg envelope as a "Dottermembran," and gives a process of development closely similar to that described for Anurida. Among insects the outer envelope is without exception, so far as determined, a true chorion formed by the follicular cells of the egg tube. The vitelline membrane which appears later is, however, a true "Dotterhaut" formed by the surface of the egg. This rule does not hold with Anurida; here the ovarian membrane is formed by the egg itself and is a "Dotterhaut," as also is the vitelline membrane which is formed after the egg is laid. Hence, in this respect Anurida resembles myriapods and crustaceans rather than insects.

It is hardly necessary to say that the paired structure of the ovary is entirely obscured in the mature stages of egg development. Eggs are crowded back into the short unpaired part and fill the body down to the oviduct. Ova are crowded above and below the alimentary tract, and no available space in the body is unused.

Ovaries after egg laying are irregular and collapsed. The only germinal cells present are in the mass of germinal epithelium. There are no large ova or traces of nutritive cells and cell masses. Whether the animals survive and lay again in the following summer or whether they die after one season has not been determined. The only evidence on the matter is this: towards the end of the summer the adult animals become scarcer, although eggs are still abundant. In the early spring, the beginning of April, there are none to be found anywhere,

while all the earliest animals to appear in the summer are smaller than those found later in the season, and some of them exceedingly small. It might be inferred from this that the old animals die and the young ones remain in the sand where they go when first hatched. This accounts for their absence in early spring and the presence of very small ones among those first found in the summer. The largest ones would be those first hatched in the preceding summer and the smallest the last; the latter have grown very little, and their chief advance from the newly hatched condition is in pigmentation. The absence of any dead bodies of the adults in the fall is easily accounted for by their exposure to the tides and consequent removal.

One more point was considered in connection with the ovarian egg; that is the existence of a micropyle. No evidence of such a structure was found; if it exists it must be extremely small. No special cells can be found taking part in its formation; the contrary is true of many of the Orthoptera, and still more so of the complicated micropyles found in Diptera, Lepidoptera, and others. Whether the places occupied by the nutritive cells would have anything to do with such organs was not decided; it seems more probable that they have some bearing on the connection of the eggs with one another. As shown in Fig. 15, *a* and *b*, the eggs are united firmly where they come into contact with each other by a thickened plate of the envelope; this is formed in the ovary, as there are no glands in the oviduct to serve any such purpose. These plates can be seen formed in unlaid eggs. As shown in Fig. 11, the follicle does not divide the eggs from each other where the nutritive cells are, and it appears that at this point the membranes are later brought into contact and firmly cemented together. Hence, the eggs must pass from the ovary in a solid, more or less irregular mass. In several cases an egg was found in the anterior part of an ovary after egg laying in an advanced state of degeneration, and the inference is that it failed to be attached to the rest of the mass and hence was not laid.

Maturation.

As has already been seen, the germinal vesicle disappears early in development, before any yolk has been formed. The nuclear wall is lost and the chromosomes are too small for recognition in the greatly increased mass of cytoplasm. From this time onward no sign can be found of the nucleus. The most careful search fails to reveal anything even in the mature ovum just before laying. No polar body spindle has been found, and the first reorganization of the nucleus making it visible is found in the section of the egg shown in Pl. XXI, Fig. 25, where the polar bodies have just been given off and the female pronucleus is returning to the centre. This egg has been laid some little time, and the male pronucleus is already at the centre awaiting the return of the egg nucleus.

Naturally Anurida is a most unfavorable form for the study of nuclear changes. Nothing determinate has been observed. Gradual shrinkage is the description best fitting the only change observable. The changes taking place in the ova, shown at *a.o.* in Figs. 10, 11, and 12, are curious, and perhaps show what occur in the others. Up to a certain stage it is impossible to tell whether a cell will develop to an egg or one of these abortive ova. Fig. 9, *o.*, may be either. There is the same reduction in the amount of chromatin, but instead of ultimately losing the nuclear membrane this swells up, enlarges, and forms a large germinal vesicle almost obliterating the cell body (Figs. 10, 13, *a.o.*). The chromosomes enlarge slightly, and, moving from the periphery, definitely arrange themselves in groups of four. This occurs at about the time of maximum yolk development, degeneration and absorption following soon after. A possible explanation of their existence may be that, although a certain number of germinal cells assume the characters distinctive of ova, still there is not nutriment enough to mature them all; hence, some are brought to a standstill in their onward progress by lack of food and undergo the degenerative hypertrophy spoken of before. The changes occurring in the nuclei of these cells may then be, in a measure, indicative of those occurring in the much smaller germinal vesicles,

and the number of chromosomes may fairly be assumed to be the same, eight, typical of insect ova.

It is common among insects for the nucleus to be so small that it can only be distinguished with great difficulty, — so much so that for a long time it was said to have disappeared altogether, and the female pronucleus appearing later was described as a new structure. This has, however, been proved incorrect, and several writers — Wheeler ('89) in Blatta and Doryphora, Blochmann ('84), Will ('85), and others — have described fully many complicated changes taking place in the germinal vesicle. It is evident from their descriptions that these changes occur later than do the corresponding ones in Anurida. In both Blatta and others the germinal vesicle remains intact until yolk formation has been almost completed; then the curious phenomena take place which render the nucleus so small; this is followed by the formation of the first polar body spindle, in which condition the egg is laid. It would seem rational to infer from this that the nucleus in Anurida exercises less control over the egg during the formation of the yolk than in the higher Insecta; perhaps this duty is performed more completely by the nutritive cells. No sign has been found of the polar body spindle nor any indication of the position of the nucleus until a much later stage than in other insects. In this respect Anurida resembles the myriapods, in many of which the germinal vesicle disappears early in ovarian development and is not seen even in the last stages. Lubbock ('61) says that in both Glomeris and Julus no trace of the germinal vesicle is ever seen in mature eggs. Zograff ('90) makes the same statement regarding the ripe ova of two species of Geophilus. Schmidt ('95), on the other hand, in describing the process in Pauropus, states that all through the period of yolk formation the germinal vesicle undergoes no change; it remains as distinct as in the younger eggs.

Comparative and General.

Passing from this brief description of the ovarian structure and oögenesis to a general consideration of the conditions of the ovary and a comparison with other forms, it is found that

Anurida differs markedly from the typical insect. The chief structural points of difference are the following:

(1) Simple paired ovaries.

(2) Absence of ovarioles.

(3) Arrangement of eggs in follicles.

(4) Presence of the germinal epithelium in two masses at the hinder end of the body.

(5) Absence of a coiled complex oviduct in which membranes, etc., are secreted.

One striking point of resemblance will be considered first; that is, the cephalic elongation shown in Fig. 1, *c.el.* This undoubtedly suggests the "Endfaden" of the ovariole, but one or two points are sufficient to show that the exact resemblance is superficial, though an analogy of function may exist. Taking the ovarioles in Blatta as typical, they are found to be numerous slender tubes, containing maturing eggs at their caudal ends, a mass of germinal cells in the middle; while the cephalic ends are prolonged into fine threads, which, uniting with threads from the other ovarioles of the same ovary, pass forward as a single strand. Heymons ('91) discusses the various theories regarding this strand, which passes cephalad and disappears or becomes attached near the heart. Müller, Wagner, and Blanchard say it is nutritive in function, an inference from its direct connection with the heart. Leydig considered that it is a membrane made of peritoneal tissue, and that it is homologous with the ovary, being formed of similar epithelial cells. Stein, Kramer, and Dufour consider the "Endfaden" simply ligamentous in function, a suspensory ligament for keeping the ovarioles in place. Heymons ('91) agrees with this view. Korschelt ('86) says that in *Dytiscus marginalis* the cell boundaries in the "Endfaden" are lost, the nuclei decrease in size away from the ovary and show evidences of undergoing indirect division, which in itself sets the cells apart from any reproductive function.

It has been satisfactorily demonstrated by Korschelt and others that the "Endfaden" is exclusively ligamentous in function; it is formed by the union of the cephalic elongations of the ovarioles; its cells have no reproductive characters. It

is clear that the cephalic elongation in Anurida is also of
the same nature; it serves to keep the ovaries attached to the
anterior body wall. Its relation, however, to the germinal
epithelium is entirely different; it is entirely separate and not
directly continuous, as in other cases. The position of the
germinal epithelium in the third and fourth abdominal segments
distinctly cuts it off from any direct connection with a strand
that originates in the first abdominal segment, apparently as a
simple continuation of the wall of the ovary, the cells of which
the two are composed being closely similar (Figs. 4, 5, *ov.*).
In Anurida as in Dytiscus, according to Korschelt ('86), the
cell outlines become indistinct and are in some places lost,
but particularly near the ovary the cells are arranged with
almost a lumen in the centre as in a duct.

The great discrepancies between the form of the ovary in
Anurida and that shown by Blatta or any other typical insect
led to a comparative study of the gross structure
of as many forms of insect ovaries as could be
reached in literature or otherwise. As regards
typical forms, Lubbock ('59) says the number of
egg tubes in insect ovaries varies from two to two
hundred (queen bees). In no case could records be
found where the number was less than two. Even
in the ant workers, according to Bickford ('95),
where the reproductive organs are practically abor-
tive, the number varies from two to fifteen, while
in the queen, the fertile female, there are forty-
five ovarioles in each ovary. Starting among the
Orthoptera it is found in the Blatta ovary that the
average number of tubes is about twenty. They
are arranged so as to open at very slight differences
of level into oviducts, which in turn unite to form
the common oviduct leading to the exterior. Fig. I

Fig. I.

in the text is copied from a plate given by Dufour
('28). It represents the ovary of *Labidura riparia*, a forficulid.
Here there are five ovarioles to each ovary. They are figured
as opening into the oviduct at different levels, making it pos-
sible to designate the ovariole as the first or fifth in a series.
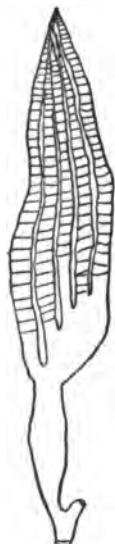
This fact is true of Blatta also, as shown by different author-
ities. The cephalic ends are united, but there is no "Endfaden";
it is quite possibly present, however. Fig. II is a copy of the
same author's representation of the ovary of *Forfi-
cula auricularia.* Here, as Lubbock ('59) says, the
ovaries consist of numerous very short tubes, three
rows in each ovary. These open into a large tube
which passes back into an enlarged chamber. The
cephalic end shows a curious elongation that clearly
unites with one of a similar nature
from the other ovary (Fig. III);
how these finally end is not figured
nor described.

Passing now to the Thysanura,
an interesting and suggestive series
can be arranged. According to
Grassi ('88), each of the ovaries of
Machilis consists of seven short egg
tubes. These open into two straight
tubes running the length of the body
(Fig. IV); according to Oudemans ('87),

Fig. III.

Fig. II.

each of these tubes is continued into a separate
oviduct, and both of these lead into a common
opening without union. There is in this stage
nothing metameric about the arrangement of the
tubules. The germinal epithelium is at the ends
of the tubes, but there is no evidence of the "End-
faden" as developed in higher forms of the insect
ovary; Oudemans ('87) distinctly recognizes its
absence. The conditions found in Japyx are some-
what similar; here there are egg tubes shorter
than in Machilis, and arranged metamerically in
the first seven abdominal segments (Fig. V); the
" Endfaden " is also absent. Campodea, shown
in Fig. VI after Grassi ('88), has a distinctly dif-
ferent structure from the two preceding Thysanura. Each of
the two ovaries has the appearance of consisting of a single
ovariole. This is, however, much longer than in the typical
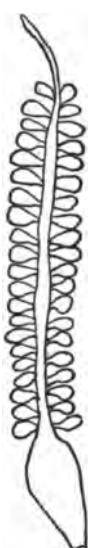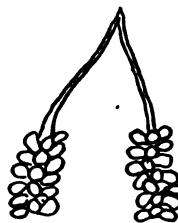
Fig. IV.

insect, as it reaches the whole length of the abdomen; the
germinal epithelium is at the anterior end just as in the typical
case.   Grassi ('88) describes and figures the ovary of Lepisma
as consisting of five tubules arranged on the same plan as in
Machilis and Japyx, differing in being distinctly metameric in
the young individual but not in the adult. *Petrobius maritimus*
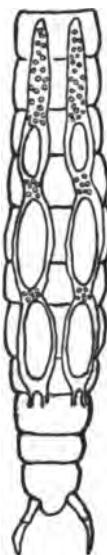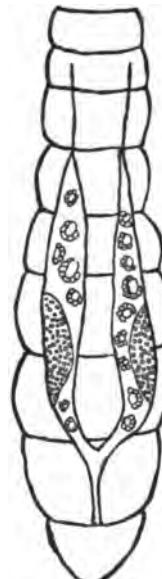
Fig. V.   Fig. VI.   Fig. VII.

is described by Lubbock ('61) as having seven short ovarian
tubes on each side of the abdomen; these tubes lie *above* the
intestine.

Passing to Fig. VII, where the conditions found in Anurida
are somewhat diagrammatically represented, there is evidently
a great divergence shown from the types already studied.   It
cannot be directly harmonized with any, agreeing as little with
Campodea as any other.   The presence of two cephalic elonga-
tions is the chief point of resemblance.   These, however, are
not connected in any direct way with the germinal epithelium;
they are apparently thin continuations of the ovarian wall.
Anurida, then, stands quite distinct in structure of the female

reproductive organs from any pterygote or even apterygote insect thus far studied. Passing down the line of tracheates the nearest related forms are among the myriapods, and here are found possible explanations for some of the peculiarities noted.

The myriapods, as a group, present so many distinct types in the structure of the reproductive organs that the validity of the group has been reasonably called into question. Without going into any discussion of the matter, a few points of comparison may be taken from among the different groups. According to Vogt and Yung ('90), the ovaries of the Chilopoda, taking Lithobius as a type, are large, unpaired, flattened, irregular masses of eggs opening by an oviduct at the hinder end of the body. They lie in the posterior part of the body *over* the alimentary canal and below the heart, while the single unpaired opening is ventral. The eggs develop irregularly in the ovary, which is rounded and widest at its cephalic end with no trace of an " Endfaden." Sections made of a just-born specimen of *Scolopendra complanata* show a median unpaired ovary lying *over* the alimentary canal and attached to it, appearing as if the cells giving rise to the ovary originated in the outer wall of the intestine. Fig. VIII is taken reduced from Fabre ('55), whose work on myriapod structure is still considered authoritative with the additional value of recent confirmation by Vom Rath ('90). This figure shows the ovarian sac of *Glomeris marginata*, a chilognath. The first impression is that this ovary is also unpaired, but, as is common with this group, this is a derived condition; the two ovaries were primitively distinct, but a fusion of their walls took place on the median line, forming a single sac.

FIG. VIII.

The ovarian sac lies below the alimentary canal, between it and the nervous system; the openings are paired and anterior,
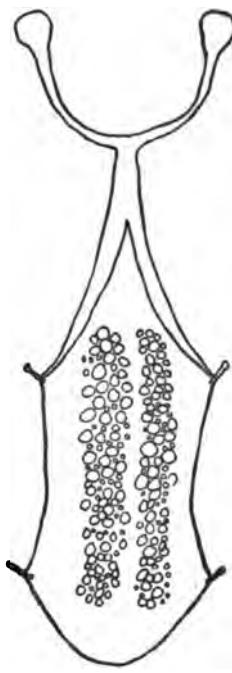
between the second and third body segments. Fabre ('55) and others describe the ova as developing exclusively on the ventral surface of the ovarian sac, which reaches back into the hind body segments. Two strings of ovules, " placentaires," extend throughout the length of the ovary, united to the membrane of the sac. Each egg is enclosed in a separate follicle, which eventually breaks, allowing the eggs to fall into the unpaired ovary, formed by the fusion of two primitively distinct sacs. Heathcote ('88) describes the ovary of the just-hatched *Julus terrestris* as an unpaired sac enclosing a double line of ovules; earlier there were two distinct sacs; a fusion followed. According to Schmidt ('94, '95), who agrees with Grassi ('86), the ovaries in Scolopendrella are paired, with paired anterior outlets opening upon the fourth segment. Each ovum is enclosed in a distinct follicle. Schmidt ('95) and Kenyon ('95) both agree in their description of Pauropus, the lowest form of myriapod; they represent it as having an ovary of the typical diplopod type. It consists of a large unpaired sac lying on the median line below the gut, crowded with ova, the largest of which are forced forward and sideways, leaving a central and posterior mass of small ova. A small unpaired oviduct opens in the third segment a little to the right of the mid-ventral line. No evidence of double strings of these ova has been observed, but only mature or nearly mature females were studied. The ova are inclosed in follicles within the ovarian sac.

Briefly summarizing the conditions found in the myriapods: in the Chilognatha the ovary is an unpaired sac, the germinal epithelium is placed chiefly at the hinder end of the body, and the developing ova pass forward through the successive segments as they ripen. The oviducts begin usually in about the fourth or fifth segment and open in paired outlets on the second or third. It is evident that a paired condition primitively existed, and that the unpaired ovary is the result of fusion of two sacs. The primitively paired condition is still indicated by the development of ova in the young animal and by the paired openings. In the Symphyla, Scolopendrella, the paired condition persists in the adult, the opening appearing on the fourth body segment. The Pauropoda show unpaired ovaries with a cephalic, asym-

metrical opening in the third body segment, and no traces of paired origin unless the eccentric opening may be such. In the Chilopoda, paired conditions are lost; no traces of them have been found; the opening is posterior and unpaired also. One more point of interest in connection with the structure of the myriapod ovary has been advanced by Lubbock ('61). He makes a fundamental distinction between the principles of follicle formation in insects and myriapods. In the latter the follicle projects *into* the ovary, while in the former it projects *from* the ovary. The importance of this distinction may not be great, but the comparisons already started between myriapod and hexapod ovaries may explain it. Comparing Figs. I–VIII the following line of development can be traced. Beginning the series with the generalized condition shown in Fig. VIII, Glomeris, and disregarding the anterior opening, Anurida is easily derived by a slightly greater localization of the germinal epithelium and non-fusion of the ovarian sacs. Japyx (Fig. V) can be derived directly from Glomeris by localizing the points of origin of ova still more sharply, and, arranging the ova in strings, by retaining a connection between the maturing eggs instead of dropping them at once into the ovarian sac, posterior unpaired openings having succeeded paired anterior ones. Machilis (Fig. IV) is the result of a condensation of the conditions started in Japyx; the tubules are elongated and crowded together. From the method of development, the germinal epithelium is at the ends of the tubules. *Labidura riparia* shows a further concentration of tubules, which in this case number only five. To gain the most highly developed hexapod ovary, it is only necessary to increase the number of tubules, which could be done primarily or secondarily. Heymons ('91), in his studies on Blatta, shows the process of origin of the numerous tubules found in the adult; from a mass of undifferentiated cells arise by rearrangement the " Endfaden " of each ovariole, as well as the common one binding the tubes together.

The conditions found in *Forficula auricularia* (Figs. II, III) form an interesting and instructive phase in the line of development. Here, according to Fabre and Lubbock, the ovary consists of very numerous short tubes, perhaps each containing a

single egg, opening successively into the ovary. This typically illustrates the difference in the position of the myriapod follicle and that of the insect, and shows the possible method of origin of the numerous tubules by progressive localization from the conditions shown in Glomeris (Fig. VIII). Another interesting point is the evidence of a cephalic elongation, which, in the case of Forficula, unites with one from the opposite side (Fig. III). Its anterior attachment was not determined.

The step from Glomeris to Anurida is short and clear; Anurida still retains the ova developing in intra-ovarian follicles, and a reduction of the germinal epithelium to a mass in the third and fourth abdominal segments is the only change excepting for the altered position of the outlet. Still further, the arrangement of the ova in strings is possibly an indication of the development of egg tubules similar to those found in the higher Apterygota.

Supposing this to be true, it is evident that the morphological value of the egg tube in Machilis and the other thysanurans is not necessarily different from that of the higher Pterygotes. Increase in number simply means arrangement in a greater number of strings, a device for accommodating more germ cells compactly in an individual. Heymons' study on Blatta strongly seconds this view. This aspect does not support the opinions held in regard to the primary metamerism of the egg tubes in Japyx. If the insect ovary came through any such series, headed by such structure as is shown in Glomeris, it is evident that metamerism, if it existed in yet earlier forms, has been obliterated; and such evidences as are seen in Japyx are either reversions or due to secondary development.

Returning once more to the series of text figures (I–VIII), there is an evident lack among the Thysanura of anything corresponding to the "Endfaden." With the exception of Anurida, there are none that show a trace of such a structure. This absence may perhaps be accounted for by the following facts. The ovaries of Machilis, Japyx, and Campodea are comparatively simple; in the first two the ovarioles are short and small and, especially in Japyx, spread throughout the body. There is not the mechanical demand for an anterior suspensory ligament

that arises on the increase in number and length of tubes found, for example, in Blatta. In origin the cells of the " Endfaden " arise from the same source as those of the ovary, and it can be considered as simply elongations of certain parts. On this basis it is difficult to see why two forms, preserving as simple an ovarian structure as that found in Anurida and Forficula, both show an anterior elongation. A possible interpretation will lie along another line. The elongation in Forficula is totally distinct from the germinal epithelium, which lies at the free ends of the short tubes. The two parts unite on the middle line (Fig. III). In Anurida the elongations are also distinct, and from their union with the fat body in the thoracic segments evidently serve the purpose of suspensory attachments. Cross-sections of these chords near the ovary show in some cases a distinct lumen in the middle of them. In some cases this persists for some distance, giving the thread the structure of a fine tube. In the embryo this part of the ovary is very striking and bends over distinctly towards the ventral wall of the first abdominal segment (Fig. 58). It appears long before any duct at the hinder end has begun to develop. Its position, structure, and relations in the adult ovary and its early development and peculiar relations in the embryo all strongly suggest a possible connection with the oviducts of the chilognath or symphylid. Change of function would now account for its relations to the second thoracic segment of the adult, but its embryonic relations suggest that it once was connected with the first abdominal segment, which bears the collophore. A suggestion might be made in regard to this problematic organ, which has been considered in so many different aspects. Wheeler ('90), following Graber ('89) and Carrière, gives these views at length, and it is only necessary to say that whichever of the three functions it serves,—of gills, sense organs, or glands,—it is undoubtedly a pair of fused appendages. In the Symphyla, which, according to Haase ('86), are nearly related to the hexapod ancestor, the genital opening lies in the median line between the fourth pair of legs (Haase, '89, Fig. 1). A study of the genital openings of Polydesmus, Craspedosoma, and other chilognaths, as given by Fabre ('55),

shows that the appendages on the segment bearing the genital opening have been more or less changed. Histologically, the condition of these modified appendages is given as being highly vascular, a description closely agreeing with the structure of the collophore in Anurida. On these grounds it is then possible that the collophore in Anurida is a relic of a former anterior opening for the reproductive organs, and that the cephalic elongation is a trace of the former duct. The exact present function of the collophore has long been a source of much conjecture, and still remains in doubt.

Fernald ('90, p. 45) summarizes the views held as to the possible present function of the ventral tube in the Collembola; two writers think it a genital organ, but its equal development in the male and female argues against this. Haase considers it a blood gill, a function he assigns to all the rest of the similar abdominal appendages found in the Symphyla, while it is said also to be a gland for the secretion of an adhesive mixture. Any of these functions may have been acquired since its original function was lost, but the evidence given above and its position on the fourth body segment — the same as that of symphyloid genital opening — give probability at least to the view here advanced of its primitive function. Kenyon ('95) gives a table of the appendages and their homologies in the various groups of arthropods, in which he considers the fourth segment of the Symphyla as the homologue of the third in hexapods, but upon what basis is not clear. Haase, Wheeler, and Grassi agree in thinking the homology of the fourth with the first abdominal correct. This view is still further supported by the suggestion of Grassi that the symphyloid ancestor of the Insecta had paired genital apertures at the hinder end of the body and also another pair between the third and fourth pairs of legs. That the genital openings among the hexapods are not very fixed in position is evident from the variety of conditions found in the different members of the group. Wheeler ('93) shows that the openings of the male and female differ, and an actual movement takes place in the former from the tenth to the ninth abdominal segment during embryonic life.

Nutritive Cells.

The possession of nutritive cells is a character widespread among the Insecta. In the majority of cases, ova are associated with a definite number of cells that assist to a greater or less degree in yolk formation and in the general growth of the ova. Korschelt ('86) divides insect ovaries into two classes, according to whether they possess these accessory cells in the form of a nutritive chamber or otherwise.   Sometimes they are arranged in separate chambers lying between the eggs, and sometimes in a terminal chamber.   Many curious forms are described by different writers.   Claus ('64), using Coccus and Aspidiotus, found "yolk strings" leading from nutritive cells to the egg, in some cases passing through the chamber of an undeveloped egg.   The same structure, a "Dottergang," is described by Wielowiejski ('85) in Pyrrhocoris.   Sometimes the nutritive cells are arranged in a highly developed follicle and absent in any other form.   Korschelt ('86) figures *Vanessa urticaria* as possessing a few such cells, closely applied to one surface of the egg, an arrangement resembling that found in Anurida.   Among the Apterygota, Grassi ('88) figures Campodea alone as having anything similar to nutritive cells; here, as shown (Fig. VI), they are interpolated in chambers between the eggs.   He expressly states, however, that the nutritive cells are *not* homologous with those of the Insecta; why he does not say, unless he wishes to consider the ovary as not the morphological equivalent of the ovariole of the higher Insecta.   In the thysanuran (*Tomoceras sp. ?*) nutritive cells were found closely similar to those described for Anurida, but smaller and arranged more distinctly as a follicle and present in larger numbers (Fig. 14).   In Anurida, as before described, each egg is associated with its string of nutritive cells, varying in number from five to eight.   Will ('84, '85) gives the results of his studies, and presents in full his idea of the compound origin of an egg, and the formation by the egg of some of the follicle cells.   As he has, however, changed some of his recent statements of the case, these studies will not be mentioned in detail.

Among the myriapods, very little trace can be found of anything resembling nutritive cells; in part they are absent, and in part too little histological study has been made of these forms. However, Schmidt ('95) describes in Pauropus a peculiar process that he interprets as the nutrition of eggs by other eggs. Ova have no definite arrangement in the hinder end of the ovary. Some cells of the germinal mass grow in size and gain yolk at the expense of the surrounding ova, which cease growing and finally degenerate. In Scolopendrella he describes an even more astonishing process in which he considers the follicle cells to be opposed to the egg cells, and *vice versa.* Follicle cells have the power of migrating into the egg cells. This migration is followed by one of two alternatives: either the egg absorbs the follicle cells or the follicle cells overpower the egg cell as phagocytes, destroying it, and afterwards wander out to become the follicle cells of a stronger egg cell. A similar case is mentioned by Weismann in Leptodora, not, however, so phagocytic in character. Yolk is formed after this battle is over, and all changes in the germinal vesicle are subsequent to this process. No mention of any such relation can be found among the diplopods or chilognaths. Stuhlmann ('86) figures eggs of *Julus sp. ?* and of *Glomeris marginata,* in neither of which are there any traces of nutritive cells.

Passing from the arthropods, Wheeler ('96) describes many forms of " Nährzellen," or accessory cells, in different groups of "worms." The ovum of Myzostoma is accompanied by two accessory cells, which gradually lose their individuality as the ovum matures. Ophryotrocha, described by Braem ('93) and Korschelt, sheds its ova into the body cavity with one accessory cell. The ovum of Tomopteris is accompanied by seven smaller cells, while in Diopatra it bears two long strings of cells attached laterally, which strings fall off before the ovum is mature. Numerous other cases are given, but these are enough to show the parallelism of development and resemblance to those found in Anurida. In the latter case the ovum accompanied by a certain number of cells is pushed away from the germinal mass of cells into the ovary, the cavity of which is the homologue in the hexapod of the annelid body

cavity. Here they are pushed forward by those developing behind, and are later inclosed in follicles made from the wall of the ovary. The difference between this follicle and that usually found in the hexapod ovary is in its direct origin from the ovarian wall, not from certain special cells in the germinal epithelium.

No further light is thrown by Anurida on the exact part taken by these accessory cells in the development of the ovum. No support is given, however, to Will's idea of the direct transformation of follicle cells into the yolk (Will, '84). Certain changes occurring in the nutritive cells previous to yolk formation and the disappearance of the material shown just preceding and during the early stages of the growth of yolk supports the view held by Blochmann ('84), Schütz ('82), and others that the nutritive cells secrete a ferment, the precursor of the yolk; this passes from the cells into the ovum and then the yolk appears. This material is probably formed also in the ovum itself. The persistence of these cells up to the time of maturity of the ovum till all the yolk is formed, their undiminished size up to this point, and their rapid degeneration afterwards, indicate an active relation between them and the egg.

The existence of lines of communication between the accessory cells and the ovum, such as was seen by Claus ('64) and Wielowiyski ('85), is evidence of a higher degree of differentiation; probably the material passed into the ovum in cases where there is a visible connection is more highly elaborated by the nutritive cells and requires less adaptation by the egg. As far as the origin of these cells is concerned, it is generally agreed that they arise from undifferentiated germ cells; it may be, perhaps, owing to a peripheral position on the germinal epithelium that more nutrition reaches them, or some other less evident cause may prevail. One thing is clear, that an added supply of chromatin is one of the first changes; from an originally small chromosome the mass increases by branching and spreading. Fine threads reach out in every direction as if to offer a larger area for contact with the nuclear plasma. It finally assumes in Anurida a strikingly stellate structure (Figs. 10, 11, 13).

Passing now to the early changes in the nutritive cells, a consideration of that peculiar structure known as the yolk nucleus is necessary.

The "yolk nucleus" is a term of varied application and, consequently, great indefiniteness. Two distinct classes of structures have been designated by this name: (1) embryonic cells concerned with yolk absorption whose origin is assigned variously to entoderm, mesoderm, superfluous spermatozoa, or the germinal vesicle, and whose fate is said to be absorption or transformation into the midgut epithelium; (2) all those structures appearing in the ovarian egg which can have but one of two possible origins: nucleus or cytoplasm of the cell itself, and whose existence may cease before the yolk appears in the egg or may be continued into embryonic life.

It is on the second of these classes that the facts observed in Anurida have a bearing. The peculiar "blue caps" that appear at a certain stage in the nutritive cells can have no other homology than the "yolk nucleus," the "Dotterkern" or "Nebenkern" of some authors.

Hubbard ('94) gives a list of groups in which the yolk nucleus has been observed to occur. This list includes all the classes of arthropods, cnidarians, nematodes, Sagitta, lamellibranchs, gasteropods, and all the vertebrate groups. The nucleus is found in eggs associated with nutritive cells and those without. Stuhlmann ('86) described it in Bombus, Vespa, Trogus, Pimpla, and Bauchus, varying in form from a diffuse peripheral mass to a localized spot. He, however, reduces the method of origin in all the Hymenoptera to one type, — that of a small concretion appearing close to or in the near vicinity of the germinal vesicle. This mass wanders away, forms a peripheral layer, collects at one pole or is scattered in several diffuse masses. Stuhlmann never satisfied himself of its nuclear origin as Balbiani ('83) had in Geophilus and Will ('84) in the frog. In all cases it is in very young ova that it appears; it becomes invisible on the formation of yolk. No mention has been made of the existence of the yolk nucleus in any other of the forms of insects. The appearance in Anurida of this blue cap in the nutritive cells declares the presence of this curious adjunct of

the ovum. It is true that no traces have been seen in the ovum itself, but the position close to the nucleus of the nutritive cells at the time just previous to yolk formation and its subsequent disappearance are all indications of its homology with the " yolk nucleus " of the second class.

Among the myriapods this body has been extensively observed. Balbiani ('83) and Zograff ('90) both report a yolk nucleus present in the small ova of the chilognath Geophilus. Balbiani figures and describes it as originating from the nucleus and in part forming follicle cells and in part the undoubted yolk nucleus, which in this case in its greatest development has a radiate structure suggesting the aster of cleavage spindles. It disappears while the ova are still very young. Heathcote observes the absence of a complex yolk nucleus in Julus such as was described for Geophilus. Lubbock ('61), describing the eggs of Julus, notes the absence of the vitelligenous bodies described for Glomeris, but notes the presence of a small body in eggs of an intermediate size that he compares to the laminated body of spiders' eggs; evidently it is a yolk nucleus. It is absent in smaller and larger eggs. Lubbock describes vitelligenous bodies as present in the egg follicles of Glomeris, but distinguishes them from yolk nuclei. Stuhlmann ('86), however, figures part of the ovary of Glomeris in which yolk nuclei are evidently present, and, from his descriptions and figures, more closely resemble those found in Anurida than any others hitherto described. Kenyon ('95) describes a small body near the nucleus in the young ova of Pauropus which disappears in older stages to be followed by yolk spherules, which is evidently the same structure, although not called so by him. Schmidt ('95) describes in Scolopendrella a small body inside of the egg cell staining as deeply as the nucleus, which he calls a migrated follicle cell, but which is quite possibly the yolk nucleus. Stuhlmann ('86) figures a large yolk nucleus present in the ova of *Peripatus edwardsii* which, strangely enough, persists late in the egg's history, even after fertilization. It is not necessary to enumerate any more forms of animals in which this structure appears; it can be said to occur among the remaining invertebrates and the vertebrates, with many variations

in its history. Usually it is present only for a short time just before yolk formation.

There can be no doubt that the yolk nucleus has an important part to play in the developing egg, and that its function concerns the formation of yolk, as agreed by Stuhlmann and Schütz. Balbiani, Sabatier, and Jatta formerly held a view regarding it as a fertilizing element, precursor of the sperm cell, but this has long been abandoned. A question naturally arises as to the origin of this necessary body, for which some name ought to be found excluding the term nucleus with which it has nothing in common, or very little, even if its nuclear origin were demonstrated. Balbiani, working chiefly among arachnids, where the yolk nucleus is largely developed, suggested its homology with the centrosome of the spermatozoan and somatic cells. Agreeing with Boveri that the centrosome has no part to play in the female cell, he considers the yolk nucleus as a case of a true hypertrophy of degeneration. It is easily supposable that in the process of degeneration some ferment should be originated useful in yolk formation; hence its preservation under a new form. Its appearance in the ovum just previous to the beginning of growth may indicate the fact that the period after the last division has taken place is when degeneration of the centrosome sets in, accompanied by its useful hypertrophy. Balbiani's figure of Geophilus shows a strikingly radiate structure in the "yolk nucleus."

The late persistence of this body in the egg of *Peripatus edwardsii* is perhaps explicable on these grounds. This species of Peripatus is viviparous, and the appearance of intra-uterine development in this form reduced the amount of yolk needed. This was consequently decreased, but as yet the reaction has not included the formative material; this is still formed, perhaps, in unreduced quantities, and hence remains not transformed into yolk. It has not yet responded to the changed conditions which require a smaller amount of yolk.

It is interesting to note that the yolk nucleus and its origin bring up the much-disputed question as to the origin of the centrosome. Balbiani's arguments demanding the origin of the yolk nucleus from the nucleus naturally carry back the

centrosome to the same source. On the other hand, Stuhl-
mann and others fail to determine the nuclear origin, their evi-
dence pointing to the cytoplasmic nature of the centrosome.
As far as Anurida is concerned, there is no clear evidence of a
nuclear origin. The yolk nucleus always appears in the cyto-
plasm close to the nucleus and on one side, but there is no
reason to think that it originates more from one than from the
other: it has more the appearance of being the result of the joint
activities of both. At least, this question cannot be settled
without determining the greater one, the origin of the centro-
some, if such an homology as has been suggested be accepted.

Unsegmented Ovum.

The eggs of Anurida when freshly laid are easily recognized
by their peculiar light yellow color and smooth, shining surface.
They are found fastened together in masses, the number of eggs
in a mass varying from a few, five or six, to at most fifteen or
twenty; each egg is about 0.27 mm. in diameter. These masses
are sometimes arranged irregularly, the eggs being simply fas-
tened together in a pile, as shown in Fig. 15, *a*; or there may be
a definite form to the mass, owing to the position of the eggs in
two rows in which the eggs alternate with each other (Fig. 15, *b*).
Very frequently in the central part of an ovum can be recognized
a lighter spot; this shows the position of the central mass of
protoplasm in which the cleavage nucleus is placed. The egg
membrane is closely adherent to the surface of the egg in these
early stages, but before cleavage takes place a distinct space has
appeared between the membrane and the egg. No further points
of differentiation are visible from the outside, but the internal
structure is marked. Pl. XXII, Fig. 30, shows a section through
an ovum having the external characters described above. The
outer egg envelope and the vitelline membrane, which is very
thin but distinctly formed by this time, are both omitted in the
figure. The egg itself is seen to be formed at this time of a large
central mass of protoplasm (Fig. 30, *c.p.*) with many radiating
strands (*r.*), which branch as they pass to the periphery, and
in most cases finally connect with a thin protoplasmic layer

that surrounds the outer surface of the egg. Between the
strands of the meshwork formed by the radiating protoplasm
lie the numerous yolk bodies (*y.*), which vary much in size.
In the central mass of protoplasm, either central or slightly to
one side, there was usually present a rather large pear-shaped
nucleus which proved to be the male pronucleus (*m.pr.*); after
entering the egg it has assumed its permanent central position
and is awaiting the return of the female pronucleus from the
periphery. In many eggs there was a smaller mass of proto-
plasm on or near the outer edge, as is shown in Fig. 30 at *p.i.*
In this is found the female pronucleus, the egg nucleus, for
the first time recognizable since its disappearance before yolk
formation began. Fig. 25 shows an enlarged view of this mass
as seen on the periphery. Large yolk bodies (*y.*) are recog-
nized imbedded in a protoplasmic mass that extends to the
surface. At *f.pr.* can be seen the extremely small female pro-
nucleus returning to the centre of the egg to meet the sperm
nucleus that is already there. *P.b.* shows the small and in-
completely separated polar bodies that have been given off by
the egg nucleus, thus completing its reduction to the female
pronucleus. Fig. 26 shows the two polar bodies at *p.b.*₁ and
*p.b.*₂; as shown, the first polar body is again dividing, giving
the typical number of three. Somewhere in the protoplasm
is the female pronucleus, but not clearly outlined. The polar
bodies never completely separate from the egg and never
clearly protrude from the surface of the egg, but remain
flattened down and are eventually absorbed into it. No sign
has been found of the polar body spindles; the egg nucleus
preserves its invisible condition in spite of the most careful
searching, the first reappearance being in the form of the
female pronucleus and polar bodies, as shown in Fig. 25.
With the gradual passage of the female pronucleus towards the
centre of the egg there is a withdrawal of the strands of proto-
plasm in the same direction, so that the central mass gradually
increases in size until at the beginning of cleavage it is plainly
visible in a fresh, whole egg as a large, lighter, central spot.
In Fig. 27 is shown a part of this mass, and in it at the centre
the two pronuclei,—the larger, the male pronucleus, pear-shaped,

and the smaller, the female pronucleus, a round ellipse. Some of the eggs contain very small central masses; several showed, instead of any central mass, a small one about halfway to the centre, distinctly containing a small nucleus. These facts point to the following interpretation: when the sperm cell enters the egg (just when this occurs was not determined) there is practically no localized protoplasm present. Its entrance presumably starts the transformation of yolk into protoplasm. During the passage of the sperm cell to the centre of the egg the protoplasm gradually accumulates, until on its arrival there it is surrounded by a conspicuous amount. Here it remains stationary until the egg nucleus, having been transformed in its peripheral position into the female pronucleus, returns to the centre surrounded by a small mass of protoplasm, the two nuclei eventually uniting to form the cleavage nucleus.

Many of the unsegmented eggs apparently undergo a process of degeneration, the process consisting chiefly in the formation of a large number of oil globules, which are protruded between the surface of the egg and the membrane; these form large masses on the surface of the egg. It is probable that such eggs failed in fertilization, and are consequently degenerating. Sections show that the yolk material is undergoing pathological changes and becoming clear in spots. The external membrane adheres more closely to the surface than in the case of the normal egg. There is, moreover, no vitelline membrane present. On a large number of normal eggs, so large a number as to suggest its constant presence, is a small raised spot, conspicuous enough to be seen on the whole egg. In section this appears as an irregular protoplasmic mass with apparently no definite structure. It possibly represents the place of entrance of the sperm cell; for a time it remains visible, but is eventually absorbed or otherwise disappears. The vitelline membrane offers no particular points of interest; it is very thin and reacts strongly to a protoplasmic stain; its color is nearly the same as the protoplasm of the outer surface of the egg.

Cleavage and Blastoderm Formation.

The cleavage in Anurida, and as far as observed in the rest of the Collembola and Thysanura, forms a striking exception to the method typical of the other Insecta. In Anurida the spherical egg has a cleavage that is slightly unequal but distinctly holoblastic. The first cleavage plane cuts the ovum into two practically equal halves (Figs. 16, 17). The second planes appear at slightly different places in the two halves and result in the arrangement of blastomeres so frequently found in annelids (Figs. 18, 19). Fig. 20 gives a different view of the 4-celled stage from that shown in Fig. 19, and illustrates the slightly unequal cleavage; this is almost a polar view and shows the shifting of the blastomeres. The second planes do not exactly halve the two parts of the egg. From this time on the planes appear regularly, the third being horizontal and the fourth more or less irregular, but in effect vertical, resulting in the 16-celled stage (Fig. 22). From this point on the details become confused by the rapid division and the difficulty of orientation. Holoblastic cleavage continues, however, up to the stage shown in Fig. 24, where a coarse morula stage has been reached. The cleavage planes are still distinct and the different cells stand up distinctly on the surface. After this, however, a change takes place that is clearly visible on the surface; the cell outlines become indistinct and the blastomeres flattened ; until after another division the surface appears almost uniformly thickly scattered with white spots that represent the nuclei and surrounding protoplasm.

A study of sections shows what has been taking place. Beginning with an egg slightly younger than that one shown in Fig. 16 the first cleavage spindle is distinctly seen (Fig. 28). The spindle does not differ in any way from the usual type. The centrospheres are represented by the darker haloes surrounding the ends of the spindle, and scattered through the protoplasm are small yolk granules in the process of transformation into cytoplasm. Fig. 29 shows the reorganization of the nucleus and the amoeboid processes of the migrating masses of protoplasm. The number of chromosomes has not

been definitely determined, owing to their extremely small size, but the indications are that there are eight in each nucleus. Fig. 31 is a section through an 8-celled egg at the line *s–s* in Fig. 21. It shows the distinct holoblastic cleavage and four of the cells. There is practically no central cavity, the cells being crowded in together. The nuclei and protoplasmic masses are already preparing for the 16-celled stage; as this division is in vertical planes, the nuclei divide in a horizontal plane and can be seen in this section. In Fig. 32, the 32-celled stage represented in Fig. 23 has been cut horizontally at about the level of the line *y–y*. This shows distinctly that the typical blastula does not result from the cleavage; some of the cells have been crowded into the interior so that a solid morula results. This condition is still more evident in sections of the typical morula stage shown in Fig. 24. Up to this point the cleavage has been undoubtedly holoblastic and practically equal, for the slight inequality shown in the 4-celled stage becomes more and more obliterated by subsequent divisions. After the morula has been formed a decided change takes place in the internal structure corresponding to the external features already described. In Fig. 33 there is shown a gradual obliteration of the hitherto distinct blastomeric outlines. The nuclei and protoplasm of the outer blastomeres have migrated to the surface, leaving the yolk masses on the inside. In the inner part the nuclei and surrounding protoplasm have entirely left the yolk masses and are evidently moving towards the surface. There has been a cessation of the total cleavage, and now the blastoderm is being formed by the migration of the cells from the blastomeres to the exterior. Consequent on this change the yolk is left behind as an inert mass; and, though at first retaining a separation into masses corresponding to the earlier blastomeres, it gradually assumes a more compact arrangement. The cells, if this name can be applied to the migrating masses of protoplasm containing a nucleus, divide as they pass to the exterior, and some remain behind, also undergoing the process of division. Fig. 34 shows the final result of this migration. Here a blastoderm has been formed by migration

from the holoblastic egg. The cells have arranged themselves in two definite layers which differ slightly in character. The outer layer is continuous, while the inner is composed of fewer cells at regular and greater distances apart. Some cells remain behind in the yolk. Following this there is a rapid division of the cells forming these two layers, until a stage shown in Fig. 35 is reached. Cell outlines become very indistinct in the blastoderm and the size very much reduced. The protoplasm becomes strongly vesicular. Some of the cells left behind in the yolk cease division at a much earlier stage and remain large, lying in the yolk; others are grouped in masses (Fig. 35, *en.*).

These are the principal steps in the cleavage and formation of the blastoderm as found in Anurida, and they can be seen to widely diverge from the centrolecithal cleavage and conse- quent migration of the cells typically found among insects. Unequal holoblastic cleavage has been described by Lemoine ('87) in Smynthurus and retarded holoblastic in Anurophorus. Ryder ('86) while describing some of the later embryological features of Anurida does not consider the early stages, so makes no note of the cleavage. Smynthurus and Anurophorus were not studied in section, so that internal changes were not described. In Anurida, as has been seen, there is a sudden change in the method of development, which results in the final formation of the blastoderm by migration, as in the case of the typical centrolecithal egg. The temporary preservation of the yolk blastomeres suggests the condition found in a centroleci- thal egg after the blastoderm is formed and secondary yolk cleavage has occurred.

Before discussing possible interpretations of the facts ob- served in Anurida, a brief sketch of some other somewhat peculiar methods of cleavage will be attempted. There is no other arthropod as yet described in which cleavage takes place in just this way. Many are holoblastic at first and change their method of cleavage during development. Among the crustaceans are found some interesting forms. Korschelt and Heider ('92) classify crustaceans according to cleavage methods, and put in the second group all those forms which start with

holoblastic cleavage and eventually lose it. Brauer's ('92) figures are given for Branchipus, in which the change takes place in the following way: holoblastic cleavage results in a distinct and regular cleavage cavity. This method continues until a layer of small, narrow, and very long columnar cells is formed, having their nuclei on the periphery and long yolk stems extending in to the centre. Eventually, this inner part forms a fused yolk mass with a single layer of blastoderm cells on the outside not separated from the yolk. Alpheus, Palaemonetes, and Hippa all have a similar cleavage according to Herrick. Ishikawa ('85) states that in a fresh-water form some cells remain behind; these are, he thinks, doubtful in significance. Among the pantopods there are two methods of cleavage. Morgan ('91) describes the cleavage in Pallene as at first total; gradually the nuclei become more and more peripheral, and eventually the cleavage planes in the yolk mass are lost. Tanystilum and Phoxichilidium present another variation; holoblastic cleavage is maintained up to the 16-celled stage with the formation of a central cavity (Figs. IX, X). This is followed by a delamination of the entoderm from the inner ends of the single layer of cells (Fig. X, *A*, *B*), filling the blastocoele with entoderm cells. In this way entoderm arises by what might be considered a process of multipolar migration.

Among the myriapods certain phases are suggestive. Zograff ('90) figures Geophilus as having purely centrolecithal cleavage of the nucleus, accompanied by a certain kind of yolk cleavage (Fig. XI, *A*, *B*). This gives the outward appearance of holoblastic cleavage that has been claimed as general throughout the group. The central nuclei now migrate outwards along these yolk cleavage lines, and the blastoderm is formed by migration. Small masses of cells are found at the ends of each cleavage line that eventually spread out and form a complete blastoderm (Fig. XI, *D*).

It is clear from these few cases that change in method of cleavage is a widespread fact among arthropods, but accomplished in many different ways. Anurida differs from any of the others described. As in Branchipus no blastocoele is formed, the strictly one-layered condition is early lost, and a

condition suggesting Fig. X, *B*, is reached (compare Fig. 32 and Pl. XXII, Fig. X, *B*). Later the relations are more like those in Fig. XI, *C*. This suggests a line of possible explanation for the conditions found in Anurida. These cells found inside the morula, as shown in Fig. 32, *c, b*, may originate by a multipolar immigration, which is the primitive method of invagina-
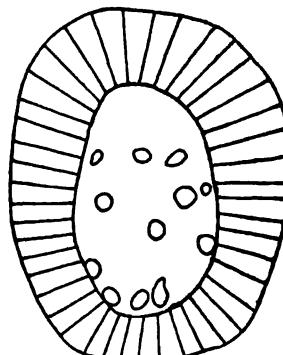
FIG. IX.
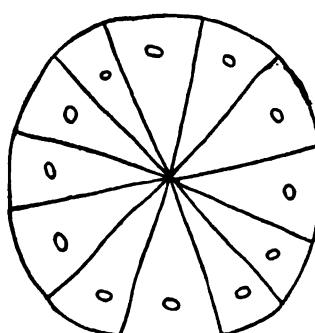
FIG. X, *A*.
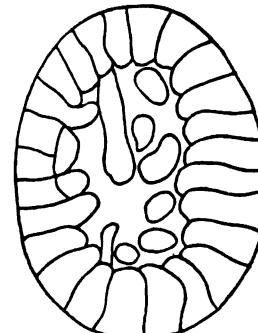
FIG. X, *B*.

tion. As the second layer of the blastoderm is formed largely by the migration of cells that were earlier inside the morula, it may be considered to arise by an imperfect and incomplete gastrulation. Whether the layer thus formed by migration from the inner part of the morula receives any additions from the outer layer is uncertain. Cells in various stages of division with the spindle axes directed obliquely to the surface point to such a possibility. The origin of the many cells re-

maining in the yolk is undoubtedly from those cells that were
inside the morula. As to the names to be applied to these
determinate layers of the blastoderm there is no doubt. With-
out hesitation the outer can be called ectoderm and the second
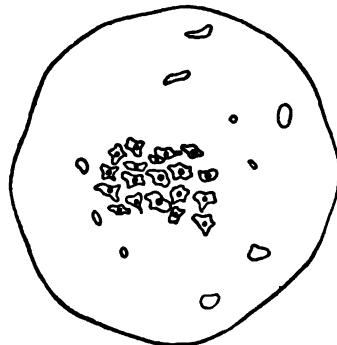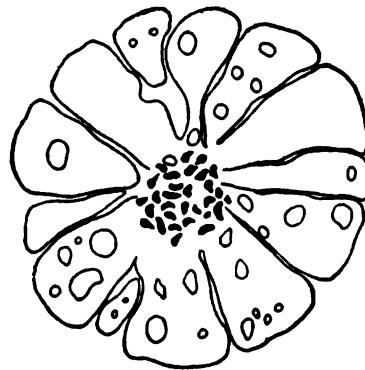
FIG. XI, *A*.   FIG. XI, *B*.
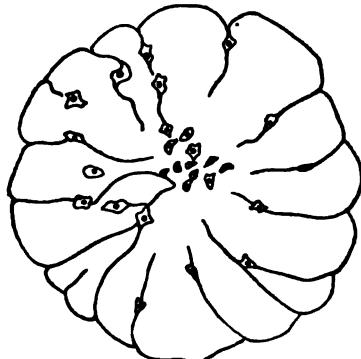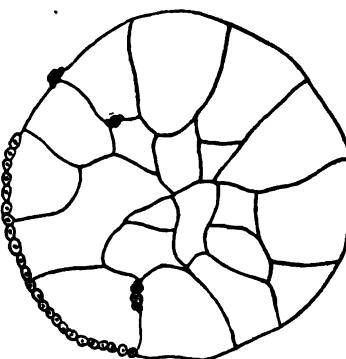
FIG. XI, *C*.   FIG. XI, *D*.

mesoderm, while, as will be shown later, the entoderm develops
from certain of the cells left in the yolk. It remains dormant,
however, until a late stage in embryonic life.

Precephalic Organ and Blastodermic Membranes.

For a certain time after the blastoderm is fully formed
further changes consist chiefly in increase in the number of
cells and decrease in their size, the lower layer always main-

taining the numerically established ratio of cells (Fig. 35). The next change is that in a certain place the blastoderm cells cease to divide, and assume certain definite characters. This is the beginning of the "precephalic organ," or, as it has been called, the "dorsal organ." Figs. 35–39 illustrate the stages by which the organ attains its full size. After the nuclei cease to divide, the cytoplasm begins to increase in amount and becomes highly vesicular in structure, forming a thick layer (Fig. 35). The nuclei increase in size, but do not divide either kinetically or akinetically. These changes apply only to the ectodermic cells. The mesoderm cells lying below these disappear gradually, partly by migration and partly by disintegration. At first the protoplasm accumulates more rapidly below the ectodermic nuclei, placing these on the periphery, but soon a process of infolding and insinking begins. The nuclei gradually sink lower as the amount of protoplasm increases, until a condition shown in Fig. 37 is reached; this is quickly followed by the stage of greatest development shown in Fig. 38. Here the precephalic organ (*pc.o.*) resembles a large gland; on a whole egg it appears as a large, circular, lighter mass that is clearly of some depth. By dissecting it out, the organ is found to have the form of an oblate spheroid, as would be inferred from its form in sections. During the development of the organ by a process of invagination, the surface over which it reaches is much reduced (Figs. 36–38), but the number of cells remains the same. The necessary crowding down of the nuclei in this process causes them to remain at different levels, suggesting that the organ is composed of several layers. This is, however, simply an appearance, there being only one layer of cells and these ectodermic. The cells always remain distinctly separated from each other, and the protoplasm is very vesicular.

The next change is a striking one; the vesicular character of the protoplasm is supplanted by a strongly marked striation that appears first at the outer edge in vertical planes. The final result of this process is seen in Fig. 39, where the nuclei are crowded to the bottom of the organ, which is irregular in shape and apparently beginning to degenerate. The outer ends of the cells have been elongated and drawn out into fine threads,

which, after being constricted to a rather narrow neck on the outer surface of the blastoderm, spread out like a fan or, when seen in the uncut egg, like a mushroom. In order that the later stages of this structure may be clear, it is necessary to consider some changes that have taken place in the blastoderm in general. The protoplasm of all the ectoderm cells has been increasing in amount and becoming vesicular, forming a deep protoplasmic layer (Fig. 37, *e.c.*) over the surface. This preparatory stage is followed by a rearrangement of the ectodermic nuclei, so as to form a wavy line (Fig. 38, *e.c.*); the protoplasm also assumes this form. No cell outlines are distinguishable; the whole blastoderm appears a continuous sheet of protoplasm, containing nuclei at certain definite intervals. After a short time, a very thin membrane separates from the surface. This is formed of thin strands connecting thickened masses (Fig. 40, Pl. XXII, *p.*). The latter are found to have come out of the troughs of the folds in the ectoderm, the thin strands from the crests. A thin layer is formed on the precephalic organ. Soon after, a layer of protoplasm is separated from the blastoderm having a definitely crenated form (Figs. 38, 40, c_1, Pl. XXII). The last part of the process is again repeated, and a second crenated membrane is formed (Fig. 39, c_2). In the region of the precephalic organ, this last membrane has peculiar relations. The elongated ends of the cells spoken of earlier are found to be directly connected with the second crenated membrane by thickened ends, which appear as a knob in cross-sections (Fig. 39, *k.*). These envelopes are developed in a way closely similar to that by which the original egg membrane is formed. The superficial protoplasm is at first markedly vesicular, and, after becoming homogeneous, is separated as a uniform layer. When the second crenated membrane is nearly formed, the ectodermic nuclei return to their original plane and the two layers again become parallel.

At the end of this process there are, surrounding the egg, five membranes. First, the egg membrane formed in the ovary but by the egg (Pl. XXII, Fig. 40, *e.m.*); then the vitelline membrane, a thin but distinct envelope (Pl. XXII, Fig. 40, *v.*). This is followed by *p.*, the material cast off preparatory to the more

complex structures following; it is very thin excepting in places where the knobs are attached, which came from the trough of the fold. The first crenated membrane ($c.1$) is the next. This is uniform in thickness and is shed over the whole surface, including the precephalic organ, which is, however, unaffected by the crenations. By the time the last envelope, the second crenated membrane, is formed (Fig. 39, $c.2$), the dorsal organ is undergoing the process of degeneration already described; its cells are elongating, the inner edge is becoming uneven, and the nuclei are shrinking to more solid masses (Fig. 39, $pc.o.$). The close connection between this organ and the envelope is readily proved by the fact that when the membrane is removed the organ is usually torn away from the embryo and remains attached to the envelope. No suggestion as to the function of the precephalic organ can be made; there was no evidence that it is particularly associated with yolk absorption; its period of greatest development precedes the appearance of the germ band. But its only obvious use begins at about this time. The ultimate fate of the structure is gradual absorption; it becomes smaller and smaller, as is seen at $pc.o.$ in Pl. XXIII, Figs. 41–45. It loses its connection with the envelope, and remains recognizable as a darker red staining mass inside the embryo, with a tuft of fine threads outside. On the hatching of the animal it is no longer visible.

From these facts it is clear that there is no structure present corresponding in origin and nature to the amnion and serosa of the other Insecta. These are distinctly cellular envelopes, and appear at a later time. The "dorsal organ," so-called among the higher Insecta, is directly connected with these envelopes. Lemoine ('87) has discussed this organ among the Poduridae, describing its form in Anurophorus and Smynthurus; he also suggests its probable relations to the structures found in the other Insecta. The organ forms a conspicuous part of the embryo in both these genera; in the former it appears early in the development of the blastoderm, but in Smynthurus its appearance is delayed until the formation of the ventral plate, and it persists until hatching. As no sections were cut, the discussion of the relations existing between this organ and the

envelopes and their probable connection with similar structures in other hexapods is somewhat vague and unsatisfactory. One point is clear, however: there are membranes in both forms that are attached to the organ, and constitute a structure important to the embryo. Lemoine says there are present at first in Anurophorus an outer thick " chorion," which is perforated irregularly in numerous places, and an inner very fine vitelline membrane. Later, more membranes are formed, the first of which appears after the formation of the blastoderm; it is described as very fine and not uniform in thickness. The author judges it to be formed of many cells identical in origin with the blastoderm cells, and calls it the amniotic membrane, naming it, however, purely from analogy of form and function. This " amniotic membrane " is connected with the dorsal organ by an ampulla, which Lemoine calls the " amniotic ampulla." Throughout the greater part of development another membrane is also present that he considers a true larval skin, as it forms on the outer parts of the appendages also.

Nicolet describes two envelopes for the poduran embryos he studied (Podura, Cyphodeirus, Desoria, Smynthurus), the outer very stiff and the inner fine, probably corresponding to the " chorion " and vitelline membrane of Lemoine. Oulganine ('75) describes two similar structures in Achorutes, Anurophorus, and Degeeria. It is evident that these two membranes are found in all these forms, but Lemoine is the only one to describe still more. Leaving out the two preblastodermic envelopes that are similarly described by all authors, one of the inner ones described in Anurophorus and Smynthurus may be considered as resembling the crenated membranes described in Anurida. This " amniotic membrane " of Lemoine is peculiar in its behavior during the life of the embryo. It possesses great powers of expansion and contraction, increasing the size of the egg by one-fourth or one-third at its largest size compared with its smallest. The author goes further and states that in contraction a folding of the surface of the embryo takes place, giving it a roughly four-sided figure with fine wrinkles over the surface.

No such powers of rapid contraction were seen in Anurida.

In living specimens the space between the embryo and the membrane is not very large, but it is practically constant; in the early stages the crenations are narrow and deep (Fig. 38, c_1), but later they are wider and shallower (Fig. 39, c_2). This is clearly associated with an increase in the size of the embryo, as measurements prove. Very soon after the formation of the membranes the first, the egg membrane, splits, and with it the vitelline membrane. Then the first crenated membrane becomes the outside cover, and a decided increase in size is observable. Growth continues until eventually the wrinkles are expanded so as to make the crenations flat in comparison with earlier stages. The attachment of this inner crenated membrane with the dorsal organ serves as a means of suspension of the embryo in the envelopes; it is thus held in a fixed position. In preserved specimens, in which some amount of shrinkage of the embryo has taken place, the space round the embryo is considerable. The latter hangs eccentrically placed, owing to its attachment to the membrane. There is one other possible use for the crenations in the envelope besides the simple one of allowance for growth. The eggs are subject to considerable variations in pressure and degrees of moisture, owing to the changes in level of the tide. The crenated surface would more readily resist the effects of this change in pressure than an unfolded one. Observations were attempted to determine this point, but nothing definite resulted, and any such suggestion must remain an inference.

It seems clear that powers of expansion belong to the embryonic envelopes of at least three of the poduran genera,—Smynthurus, Anurophorus, and Anurida. As regards the causes of such changes, there is less known; in Anurida, growth and possibly changes of pressure are the direct agents, while in Anurophorus and Smynthurus contraction and expansion take place regularly without any apparent external or internal cause. Lemoine's Fig. 16 strongly suggests another interpretation of the crenations found in the embryo; it so much resembles the early crenated stages of Anurida as to make it possibly a corresponding stage, instead of an embryo undergoing excessive contraction.

Leaving the podurans, there are points of interest to be found in connection with the higher groups. Wheeler ('93) discusses a curious structure found in Xiphidium, which he calls the " indusium." It appears at the same time as the germ band, or a little later, and is ventrally placed on the long oval egg, just in front of the head. It remains unchanged for some time, usually separated from the head, but sometimes connected by a small string of cells. Ultimately by proliferation it forms an envelope, pushing its way between the serosa and the yolk, and finally becomes an inner membrane next to the yolk, and only separated from the embryo by the amnion. Strange to say, this organ forms for itself an amnion, which spreads round the egg and is recognized as the outer " indusium." The author homologizes this with the poduran " micropyle," and seconds the previous suggestion that the latter is truly homologous with the " dorsal organ," as found in some groups of the Crustacea.

Among the Crustacea there are found curious intermediate structures. Bobretsky ('74), in his studies of Oniscus, which have been corroborated in part by Nusbaum ('86), discusses the so-called " primitive cumulus " or " dorsal organ." Its origin as described is similar to that of the precephalic organ in Anurida both in manner and in time. Excepting in the dorsal half of the embryo the two germ layers are distinct. After remaining stationary for a long time the cells increase in number and spread out over the dorsal part of the embryo as a cap. This cap is connected with a thin membrane that the author calls a larval skin. At the greatest development of this organ it remains as a saddle-shaped cloak composed of a single layer of cells.

In embryos of two species of Idotea found at Woods Holl, a structure similar in its early stages to that of Anurida was found, but it was paired, one small organ being placed on each side of the middle line. This resembled closely Nusbaum's ('87) figures of Mysis.

To make a graded series between Anurida, Oniscus, and Xiphidium is easy. In the first the organ is large, active, and functional in very early stages; it later begins to degenerate and assumes certain secondary characters, as, for example, the

connection with the membranes. In Oniscus the cells migrate bodily instead of simply elongating, and form a cellular cap instead of a membranous one. In Xiphidium the cellular envelope is completed and entirely encloses the embryo. Wheeler's suggestion is that the organ he calls the "indusium" had probably lost its original function, and was degenerating and varying in consequence; accidentally acquiring a new value, it was reconstructed for its new use as an embryonic envelope. In Oniscus this process of reconstruction is not yet completed, and in Anurida barely begun. There is an interesting suggestion in Wheeler's ('93) mention of the embryonic sucking disc in Clepsine. A complete series may possibly be made between this disc, the organ as found in Anurida and the phyllopod cervical gland which actually functions as a sucker, and is regarded by Müller ('64) and Grobben ('79) as the homologue of the "dorsal organ" of the Amphipoda. In this case the power of adhesion that still belongs to the precephalic organ in Anurida is possibly a remnant of its former function. The gradual prolongation of embryonic life causes the young to hatch in a more mature stage, and need for the adhesive disc of the immature larva is lost.

Embryo Formation.

After the separation of the second membrane the formation of the ventral plate or germ band begins. On surface views it first appears as a narrow band passing round the egg in such a way that it nearly encircles it, the precephalic organ being the separating mass. The head of the embryo lies on one side of it, and by crossing the organ the tail is found at the opposite side (Pl. XXIII, Fig. 41). Almost immediately the outlines of the embryo can be distinguished, the different parts being laid down successively from the head backwards. The mesodermic somites indicating the future segments of the body appear, and almost at once the appendages of the different parts. In as early a stage as that shown in Pl. XXIII, Fig. 41, the beginnings of the antennae, mandibles, maxillae, and thoracic legs are evident, and Pl. XXIII, Fig. 40, shows an added pair of

appendages between the antennae and the mandibles, as well as the faint outline of the rest of the germ band. Up to the stage figured in Fig. 42 the chief changes are in the clearer definition of the six abdominal segments, the appearance of the median unpaired labrum, and the indication of the procto-deal and stomodeal invaginations. The antennae have become undoubtedly three-jointed, with an indication of a fourth, and the precephalic organ has begun its process of elongation and degeneration (Fig. 42). The embryo still preserves its spheri-cal form, and stretching across between the ends of the appen-dages can be seen the last envelope formed (Figs. 42, 44, *m.*). Whether this is a true larval skin or is similar to the "Blasto-dermhäuten" already discussed is not clear, but it can best be seen after the appendages have appeared. It seems most likely that it is shed just at the beginning of the embryo formation; it passes round the embryo, and is frequently found attached to the ends of the precephalic organ.

From this point a radical change of form takes place; a flexure of the embryo begins that results in crowding the mouth-parts together to form a definite head and folding the embryo upon itself. This greatly changes the points of refer-ence in regard to the precephalic organ: the head remains in about the same position, but the tail is drawn much farther away, and the embryo becomes restricted to less than one-half of the circumference of the egg, instead of extending over nearly the whole. At the same time there is a marked lateral flattening, so that the young animal is much narrower meas-ured from side to side than measured dorso-ventrally. Before the final stage of this process is reached, however, certain features of note have appeared. The most striking of these are shown in Figs. 43 and 45. Fig. 43 represents an embryo in which the ventral flexure has just begun, as shown in the side view of Fig. 43, *a.* The brain lobes, the protocerebrum, have clearly appeared, and their elongation into optic lobes is evident. The labrum, unpaired, and lying on the middle line, is seen just anterior to the stomodaeum. The antennae lie on each side of the future mouth, and are formed of three short, thick, and approximately equal joints. The three pairs of

mouth-parts are next in succession. On each side of these has appeared a ridge that passes backward along the embryo, the two folds enclosing the mandibles and maxillae. These folds start from just the region where the small intercalary appendages were seen earlier, but which have now disappeared. Figs. 43, 46, and 47 show the process by which this change takes place, and leave no doubt that the folds as they finally appear are a development from the intercalary appendages. This sheath-like form of the extra mouth-part explains the well-known peculiar structure of the adult head. The adult mouth has always been described as deeply sunk into the head and appearing as a tube, out of the end of which the points of the mandibles and maxillae protrude. It can be readily seen that the labrum in front and these lateral folds make together a three-sided box in which the mouth-parts, two mandibles, and four maxillae are sheltered.

In Fig. 43, where flexure has just begun, the thoracic appendages are visibly longer and more distinctively legs. The first abdominal segment bears a large pair of appendages that are ultimately modified to form the collophore, while on three of the succeeding abdominal segments there are also small appendages, those on the fourth segment being the largest. This condition is equally evident in Fig. 44, a slightly later stage. In Fig. 45 the conditions are still the same; the collophore is, however, almost hidden by the flexure of the body, and the terminal segment has elongated into two decided folds that surround the proctodaeum. The five single eye-spots have appeared on the sides of the head, and the precephalic organ is much reduced and shows the thread-like elongation of its cells.

An interesting question is raised by a consideration of the folds that rise round the mouth. The simple structure of the adult mouth in these forms has been discussed by Fernald ('90); he describes it as being a pouch of considerable size, at the inner end of which are attached two pairs of jaws; these are entirely enclosed. He cannot, however, determine the exact homologies of the different parts. Hansen ('93) discusses the homologies of the mouth-parts of the Crustacea and Insecta by studies on Japyx, Campodea, and some of the Collembola. He finds it a

common peculiarity that the mandibles and maxillae are sunk deeply in the head up to the points, as in the case of Anurida. He speaks of a fold of the skin that causes this insinking which is attached to the labrum, and is undoubtedly similar to the fold found in Anurida. Campodea, Japyx, Machilis, and Lepisma all agree in general details, but the last mentioned is considered by Hansen a transition form between the Thysanura and the Orthoptera. The relations as seen in the anuridan embryo are as follows: The unpaired labrum forms the upper part, the front of the pouch, at the back of which work the two pairs of jaws, the mandibles, and the first maxillae, while the second pair of maxillae has been modified to form the back of this pouch. The lateral folds already described make the sides and are developed as shown from the intercalary segment.

The question naturally arises as to what homology this additional pair of mouth-parts can have, arising as it does on a distinct segment. Viallanes ('91) and Wheeler ('93) agree in the following structure of the orthopteran head and brain: It consists of a protocerebrum, the most anterior segment, forming the mass of the supra-oesophageal ganglion, from which the large optic nerves are developed. A deutocerebrum and tritocerebrum follow, which together complete the brain and the oesophageal collar. Following this is a series of ganglia corresponding to the mouth-parts, which eventually fuse to form the suboesophageal ganglion of the adult. These authors find distinct mesoblastic somites in the segments of both the deuto- and tritocerebral lobes, and hence conclude their equivalence in value to any of the succeeding segments. The antennae of insects are enervated from the deutocerebrum, and, as has been demonstrated by Viallanes ('91) and St. Remy ('90), the first pair of crustacean antennae is also connected with this brain lobe, the second pair being enervated by the tritocerebrum. Hansen homologizes the mandibles in the two groups, but does not decide on the antennal homology. It would seem clear from the work already mentioned on the brain that the homology of the first antennae of the Crustacea with that of insects is practically decided. Arguments drawn from the absence or presence of either pair of antennae in the higher

Crustacea are not convincing, as there is great variation in the degree of development of these appendages in different groups. In some the first antennae are larger and the second small or absent, and in others the reverse is true. The evidence from the lower forms is more reliable. Ray Lankester enumerates the different appendages found in that archaic type Apus, and indicates that the first antennae are always present while the second are sometimes absent and sometimes present, in the same species, and always missing in some species. As Apus is considered more generalized in its structure than any other crustacean, it is suggestive that the first antennae should be constant and the second the more variable. This immediately suggests an interesting explanation for the added pair of mouth-parts found in Anurida, originating from the tritocerebral segment. On this basis they are a modified form of the second pair of antennae in the crustacean; and hence Anurida, including, without doubt, its allied forms, possesses an adult structure clearly homologous with the second antennae, the very important appendages of some crustacean heads. It is interesting in this connection that Hansen considers the musculature of the head of Machilis much more like that of the crustacean than that of the insect.

Fig. 48 represents an Anurida just hatched. It can be seen to have many of the characters of the adult form; it is, however, perfectly white, showing none of the black pigment characteristic of the adult. The surface of its body is not as wrinkled and folded at this young stage, and the cuticle lacks the finely papillose surface found in the older specimens. The antennae are clearly four-jointed, as described by Ryder ('86); the terminal joint is less pronounced, however, and sometimes is not completely separated from the third. Instead of a third joint there exists only a constriction. The collophore is prominent; it originates by the fusion of the two appendages on the first abdominal segment. Young animals at this stage are very active, and may be found in large numbers in the same places as the eggs. Judging from the great variation in the size of the eggs at the time of hatching, there is a great variation in the size of the animal; this must be the case,

because it is not a rule that the smallest is the least developed. Quite often the smallest ones have undergone considerable post-embryonic development, while some larger ones are much farther back in the process. Pigmentation and increase in size are the chief external changes that are needed to make the young Anurida resemble the adult. Both of these characters come slowly, though the young are probably all pigmented by the end of the season. They remain small in size, however.

Comparing these results with Ryder's ('86) figures, which are, as far as known, the only published studies of the embryonic stages of Anurida, certain differences are observable. There are figured in these the two crenated membranes and the early stages of the germ band, several later embryonic forms, and young and adult animals. The chief difference in the embryos as figured by Ryder and those shown in Pl. XXIII of this investigation lies in the different interpretation of the embryonic head appendages. Ryder recognized but three pairs: one pair of antennae, one pair of mandibles, and one pair of maxillae. He included the second maxillae with the thoracic legs, and did not see the intercalary appendages. In the recently hatched young he describes a structure placed on the anterior part of the fourth abdominal segment which he considers represents a rudimentary spring. No evidence of such a structure was seen in the young investigated, and large numbers were examined. In the embryonic stages the appendages on the fourth abdominal segment are larger than any of the others, excepting those on the first (Fig. 45, *a.*4); but these, like all the others excepting those on the first, disappear before hatching.

The process by which the germ band arises is exceedingly simple. Immediately after the formation of the second crenated membrane, or even before, or in some cases before all the entodermic nuclei have sunk to a common level again, the mesoderm cells may be seen migrating to such a position that one meridian passing through the precephalic organ and the centre of the egg would cut the band they form longitudinally into two. The migration eventually leaves the greater part of the egg covered only by ectoderm and the germ band appears

girdling the egg. At first one-layered, the mesoderm early shows a further change to two in certain parts which represent the mesoblastic somites. Fig. 49 represents a cross-section through the germ band just after its formation. In the middle line, under the median ectodermic depression, the mesoderm is a single layer of cells; but on each side there are the early indications of the somites. The two-layered condition arises by migration, and the cavity when present is hollowed out afterwards. Subsequent modifications arise by differentiation from this primitive condition.

Origin and Development of the Entoderm.

The place and manner of entoderm formation long remained in doubt, as the appearance of the mid-gut is delayed till very late in embryonic life, and these late stages are difficult to find. However, the following facts and explanation were eventually determined. By the end of cleavage two definite layers are fully established, the ectoderm and mesoderm, as shown in Fig. 35. There are, moreover, certain cells left in the yolk that have never taken part in the formation of either of the two layers. Some of these that are spread singly through the yolk are evidently yolk cells, and function in the transformation of yolk for the nutrition of the embryo (Figs. 35, 37, 39, *y.c.*). In addition to these, however, there are some cells that remain grouped in clusters, the whole mass evidently arising by division from a single cell or perhaps a few cells. The clusters are placed above the centre of the egg, using the precephalic organ as the pole of the reference axis, and very frequently limited in numbers to two (Fig. 35, *en.*). One of two things now happens to them: they either migrate from the masses and are scattered through the yolk in small groups of twos or threes, or else they remain unchanged for a considerable length of time. In the former case they are difficult to distinguish from the yolk cells, but their greater transparency, larger vesicular cell bodies, and association in small groups is a sure guide to their identification (Pl. XXII, Fig. 41). The yolk cells early acquire a more deeply staining nucleus, showing the characteristic increase of chro-

matin in cells with a strongly assimilative function. In the second case, when these cells remain permanently associated in one or more clusters, no further change occurs until late in embryonic life. By the subsequent development of the embryo and its changes in form during flexure, the relative positions of the groups are somewhat changed. One large mass may, however, readily be recognized in the region of the proctodaeum, not far from the ectodermal layer.

After the embryo has reached a stage corresponding to Fig. 45, or perhaps later, the mass may be seen to be scattering, and certain changes occur in the yolk. Around some cells are large spherical masses of yolk particles contained in extremely vesicular protoplasm, in which there is a small central nucleus (Fig. 58, *en.*). These are particularly numerous in the regions of the stomodaeum and proctodaeum, and may be clearly seen later to assume a regular arrangement in the yolk, forming two broken lines reaching through the body. It is now clear what these mysterious cells are : they are the entoderm, and are taking up definite positions to form the mesenteron of the young animal. In a newly hatched specimen an interesting relation is shown; Fig. 65 represents part of a frontal section through the body of such an animal. The mesenteron is seen to be composed of large irregular-shaped cells with extremely vesicular protoplasm; the nuclei are irregular in size and stain faintly. At the inner or free edges of the cells masses of yolk are visible, and certain of the cells also contain similar particles. Whether these particles are passing out of the entoderm cells to the enteric cavity or are being ingulfed by them is not clear, but in either case it is evident that the mesenteron when fully formed contains very little food yolk, a condition contrary to the general rule. The entodermic cells are resting on an extremely thin membrane, but there is as yet no sign of muscular walls or other differentiation. The cells are themselves still irregular and almost amoeboid in form (Fig. 65, *en.*).

This is, then, the history of the entoderm in Anurida; it originates without doubt during cleavage, and takes up its position in the middle of the morula by a process which it is possible to call invagination. When the mesoderm, which also lies within

the morula, migrates outward and forms a definite layer below the ectoderm, the entoderm remains in the interior as one or more cell masses and is comparatively unchanged till a late period of embryonic development. Finally the cells separate and increase in size, and ingulfing yolk arrange themselves to form the definitive mesenteron, which contains practically no yolk excepting some in an intracellular condition. Whether the vitellophags are genetically connected with entoderm is not clear, but they very possibly are entoderm cells that early assume their digestive powers. That they do not, however, take part in the formation of the mesenteron is clear from their presence at the time of its formation scattered throughout the yolk, recognizable as shrunken degenerating bodies (Fig. 57, *y.c.*).

At first sight this process is markedly different from that described for other insects; but the differences admit of quite ready explanation. The typical process of entoderm formation in the other Insecta is by proliferation from two formative centres, an oral and anal, that appear at the two ends of an elongate blastopore. This process has been demonstrated for the Coleoptera by Heider ('89) and Wheeler ('89); in the Diptera by Voeltzkow ('89) and Graber ('89); in the Hymenoptera by Carrière ('90); and in the Orthoptera by Wheeler ('93). In several other forms but a single formative centre is described, that one being the anal. From this one or these two centres a continuous band is formed by proliferation that finally incloses the yolk completely. The great difference between the two processes, as described for Anurida and the rest of the Insecta, lies in the different disposition of the yolk; in the former case it is not inclosed in the mesenteron, and in the latter it is. The other variations may be harmonized in the following way: The groups of cells that are usually two in number can be considered to be the equivalent of the two masses in the higher Insecta; the difference in size is marked, the anal mass being the one more likely to persist in a recognizable condition. A similar difference was observed by Wheeler ('93) for Xiphidium, where he found the anal centre decidedly larger than the oral. The position of the two masses or, as it may be, one rather scattered mass just below the precephalic organ may be indica-

tive of its future anal position, although at this time the germ band is not yet laid down. By a somewhat early migration the entoderm cells are scattered, and finally assume their definite relations at a very late embryonic period. It is, of course, possible that the entoderm cells even at this stage assist in the transformation of the yolk, but there are certainly separate yolk cells for this duty.

As readily seen, the process of entoderm formation in Anurida agrees very closely with the method found among some of the Crustacea. In many members of this group the entoderm is early differentiated from the rest of the cells, but remains stationary for a long time, simply imbedded in the yolk. In some cases its origin is still under discussion; some authors claim that it is composed of vitellophag cells that have been functioning in the egg from the beginning; in many cases the entoderm only assumes its permanent relations at a late period of development. Zograff ('90), describing the origin of the mesenteric lining in two species of Geophilus, says that it appears from the yolk, and concludes that the process closely resembles that found in Malacostraca and Arthrostraca. Heathcote ('86) describes it for *Julus terrestris* in the following way: Certain cells during cleavage remain behind in the yolk and form the entoderm, and in turn give rise to the middle germ layer. After the appearance of the ectodermal parts of the alimentary tract the scattered entoderm cells arrange themselves to form a central lumen and give rise to the mesenteron. From these few points of comparison it is clear that Anurida constitutes an interesting intermediate form, connecting the processes typical of the crustaceans and myriapods with those of the higher Insecta. It is difficult to say to which of the lower arthropod groups Anurida is the more closely allied, especially as so little work has been done on the myriapods. Certainly the resemblances to the group last mentioned are very striking. The interpretation of the differences found between the higher insects and Anurida would point to a gradual delaying of the entoderm formation to a later embryonic period in those eggs possessing a larger quantity of yolk. This new relation possibly raises again the question as to whether all the cells resulting from cleavage of

the central nucleus in centrolecithal eggs pass to the surface
to form the blastoderm. Some may remain behind and form
the vitellophags and perhaps take other part in development.
It is certain in Anurida that vitellophags do not migrate out-
wards and then return, but are left in the yolk.

Development of the Reproductive Organs.

The development of the germ cells was found to be one of
the most interesting processes followed in detail. Their appear-
ance takes place at a comparatively late period of embryonic
life, the earliest stages occurring when the animal has reached
the stage shown in Fig. 44.[1] At this period the processes rep-
resented in Figs. 50 and 51 are seen to take place in the second
and third abdominal segments. Both these views represent
cross-sections of the mesoblastic somites of one side of these
abdominal segments, and show the great variation that occurs
in the distinctness with which the cavities of the somites are
developed. In Fig. 50 two germ cells (*g.c.*) are seen, one pass-
ing out into the yolk on the free side of the somite, and one as
yet imbedded in the splanchnic layer of the mesoderm. These
cells are readily recognized by their peculiarly clear transpar-
ent cell bodies. In Fig. 51 is shown another stage, when the
germ cell is clearly inclosed in the cavity of the somite. Figs.
52, 53, and 58 show the line of development followed in this
latter case. The germ cells are distinctly between the walls of
the two mesoblastic layers, the splanchnic and the somatic. In
Fig. 53 a definite form has already been attained by the germi-
nal mass. There is a cephalic elongation and a hinder spherical
mass. The surrounding mesoderm has been differentiated into
muscles, and connective tissue is beginning to appear. There
is, however, a distinct layer of mesoderm separating the germ
cells from the yolk, the splanchnic layer; the germinal mass
lying in a space appearing to be a true body cavity. In Fig.
58 the mass of cells is much larger, and by the crowding
together of the abdominal segments it can be seen that flexure

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[1] Since the reproductive organs are paired and the process is similar in general
principles, descriptions will be made of but one side. The only difference lies in
a slight variation in position of the two organs.

of the embryo has proceeded much farther. The continuous mesoderm sheet separating the germ cells from the yolk mass has been broken; at the most curved part of the mass of germinal cells there is direct communication between them and the yolk. Whether this breakage is due to rapid flexure or rapid increase in the number of germ cells, which show evidences of frequent division or to both causes, one thing is clear, that the germ cells are now in close contact with the yolk.

Returning now to a consideration of Fig. 50, the fate of germ cells originating in the second way may be seen in the series shown in Figs. 50 and 54–56. The single cell, set free on the outer side of the somite, increases to an irregular mass that lies in part sunk into the mesoderm and in part projecting out towards the yolk. These cells, at first a solid group, migrate outwards and begin to mingle with the yolk (Fig. 54, *y.*), the migration being most noticeable in the outer cells, those nearest the yolk. In Fig. 55 migration has not gone so far and the greater magnification shows the peculiarly "succulent" character of the cells. In Fig. 56, illustrating the extreme result of the process, the cells have divided into two groups, one (*s.g.c.*) remaining near the mesoderm and by repeated divisions increasing to a large mass of small cells, another (*m.g.c.*), which has migrated out and is spreading through the yolk, still maintaining, however, a certain relation to the stationary cells. Unfortunately, in spite of the most careful search, the latest embryonic forms found do not seem to supply the next step. The final result is seen in the figures in Pl. XXV, Fig. 64 concluding this series. This represents a longitudinal slightly oblique section of a just-hatched animal, showing the reproductive organs of one side of the body. At *g.e.* is a somewhat irregular mass of cells forming the germinal epithelium, lying in the second and third abdominal segments. Below this, and directly connected with it, is a large irregular sac filled with yellow material, in which are scattered a few large cells. Two lobes of this sac are cut through, and at its lower end, coming from the hinder end of the fifth abdominal segment, is an ectodermic invagination, the duct of the reproductive organs leading to the exterior. This animal is recognizably a young male, the parts described

corresponding to the parts of the adult. The yellow material in the sac-like parts of the organs is yolk (Fig. 64, *y.*), and the golden yellow globules scattered through the connective tissue is yolk acquiring the characters of fat globules. The whole body is very simple in structure, a few muscle fibres, the ventral nerve chain, and a large amount of connective tissue filling up the space being the essential elements; numerous blood corpuscles loaded with yolk present in the small body cavity were not represented in the figure. The reproductive organ is shown at a later stage of development in Fig. 65 in frontal section; the yolk has been absorbed from that part of the sacs near the germinal epithelium, and by rapid proliferation, probably rendered possible by the ample supply of food, very small sperm cells are being formed that will eventually mature as spermatozoa; these fill the sac.

In Figs. 59, 60, and 62 the story of the first group of germinal cells originating within the cavity of the somite is continued. Fig. 60 represents a cross-section of an ovary from a recently hatched or at least very young Anurida. The cut does not include the germinal epithelium, but some of the cells that have become detached from it are figured that show characters rendering their recognition easy. At *n.c.* are cells that bear all the distinctive marks of nutritive cells, large nuclei, richly supplied with chromatin, which is irregularly massed together, but not stellate in arrangement. At *o.* are seen cells that as distinctly possess the characters of ova, large cell body, and small clear nucleus. Scattered among these are granules of true embryonic yolk of irregular sizes. This is even included in some of the ova, as shown in Fig. 59. The ovarian wall is extremely thin; small nuclei occur at intervals that closely resemble the mesodermic nuclei of younger stages. Fig. 61 is a representation of an ovum with its nutritive cells, as found in an animal taken early in the summer, evidently before development had begun for the season. This shows how little, excepting in one respect, the ova and nutritive cells change during the winter. The one respect is in the chromatin of the nutritive cells; in the last figure the definite stellate structure is attained, while in the younger forms the chromatin

is in coarse threads (Fig. 62) or irregular masses (Figs. 59, 60). In the size and external characters of the animal the changes in the interval are marked, but practically no development has taken place in the reproductive cells. The external part of the reproductive organ, the outlet, was found to originate in the late embryonic stages by a median unpaired invagination from the hinder end of the fifth abdominal segment. As shown in the adult, the ectodermic part of these ducts is extremely short, being only the small unpaired part extending from the exterior through to the body space, where it joins the mesodermic part of the organ. This is found completely invaginated in late embryos, and showing in the female the accessory diverticula, the receptaculum seminalis, as a branch of the main duct.

When it was found that the yolk was not contained in the mid-gut of the embryo, the question naturally arose as to its final disposal. It has been seen from the above facts that a large part of it is included within the reproductive organs, and serves to hasten very much the maturing of the generative elements. A very large quantity is also found in the blood corpuscles of the newly hatched animal. Fig. 63 shows some of these taken from the same animal as Fig. 59. Gradually during their circulation through the body they must give up their rich supply of food. No complete observations were made on the origin of these cells, but it is probable that they arise from the mesoderm. Already in Fig. 53 small isolated meso-dermic cells can be seen, and many are found later in different parts of the body. Even when loaded with yolk there is no possibility of confusing them with yolk-laden entoderm cells, they are so very much smaller in size (*cf.* Fig. 63 and Pl. XXII, Fig. 41 ; 63 is magnified more than 41). Yolk was found in the places already enumerated and also free in the body cavity, lying chiefly under the alimentary canal. Thickly scattered through the meshes of the connective tissue are many yolk spheres, which are eventually transformed into fat globules. When first hatched, the body cavity, as it is called, or, more correctly speaking, the haemocoele, is much obscured by a large amount of connective tissue that originates from the mesoderm. The alimentary canal, reproductive organs, and nervous system all

lie more or less imbedded in it, and it is only by later post-embryonic development that the space is finally cleared and becomes as distinct as in the adult.

The history and fate of the slight trace of the true coelom, as seen in the female embryo, has not been studied in detail and must remain a point for future investigation. As seen in Fig. 51, there is a distinct cavity in the mesodermic somite, although this is not so clearly marked in all the segments. In Fig. 58 the splanchnic layer of mesoderm forms one side of a spacious cavity, evidently resulting from the fusion of those parts of the somite cavities not cut off in the appendages. This space is a striking feature in animals of this sex and renders them immediately recognizable. It is, however, eventually obliterated; the beginning of this process is shown in the breaking of the splanchnic layer, thus allowing the germinal mass to leave the distinct true coelom. It is curious to find so much more reduction in the coelomic space in the male; it is as noticeably absent from the beginning as it was present in the female. At all times the germ band remains a solid mass; no space such as is shown in the female is ever seen.

The loss of the coelomic cavity is without doubt a derived condition, as the ancestors of the insects probably inherited the space more or less completely from their annelid-like progenitors. The retention by the female of primitive characters not found in the male is a frequent occurrence, the latter sex being the more subject to modification. The female is the more conservative and adheres more closely to the primitive type.

This inclusion of yolk in the reproductive organs is a point of great interest in the development of Anurida. It is a phenomenon not frequently observed in any forms, and, as far as known, without a direct parallel among the Insecta. There are certain forms, however, in which resemblances may be noted. Metschnikoff ('74), in his classic account of the embryology of Polydesmus and Julus, double-footed myriapods, speaks of the peculiar position of the nutritive yolk in the body of the young embryos. It is present almost exclusively in the body cavity; very little is found in the intestine. This is a fact true also of the daphnids, where the yolk lies in the body cavity between a

yolkless alimentary canal and the remaining viscera. Mordivilko ('95), discussing the structure and development of some of the aphides, speaks of Metschnikoff's " secondary yolk " (Metschnikoff, '66), and shows how it lies in the body cavity surrounding the reproductive organs and causing a wonderfully rapid growth on their part. Among the vertebrates certain forms exist in which a quantity of the embryonic yolk is associated with the germ cells, causing their rapid growth. Petromyzon, the lamprey, belongs in this category, and, as shown in Fig. 66, much of the yolk is included among the germ cells.[1]

Summary.

Summing up the results of this investigation on Anurida the following points are of interest:

(1) That the ovary is very simple in character, no arrangement corresponding to the ovariole of the higher hexapods being present.

(2) A long anterior elongation is present, composed of cells non-germinal in character and serving as a suspensory ligament. Homology with the "Endfaden" is uncertain.

(3) Ova are associated with nutritive cells that show distinct "yolk nuclei" at a certain stage.

(4) The germinal vesicle early becomes invisible and the nucleus does not again appear until after the polar bodies are given off.

(5) The egg is spherical, cleavage holoblastic at first and slightly unequal.

(6) There is a multipolar immigration suggesting gastrulation.

(7) Outer and middle germ layers are formed by migration, the entoderm remaining behind in the yolk with yolk cells.

(8) A precephalic organ homologous with the dorsal organ of some crustaceans, and the indusium of Xiphidium is developed in the early blastoderm stages.

(9) There are at least three cuticles formed during preblastodermic stages; two of the three are crenated.

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[1] This figure is from an unpublished drawing of Dr. W. M. Wheeler, who kindly lent it for this purpose.

(10) The embryo appears encircling the egg as a narrow girdle, stopping each side of the precephalic organ.

(11) An extra pair of mouth-parts appears, forming in the adult two lateral folds inclosing the mouth-parts. This is homologous with the second pair of crustacean antennae.

(12) Yolk is included in the reproductive organs and lies free in the body cavity, but is not found in the mesenteron.

(13) Anurida shows characters allying it with crustaceans and myriapods rather than the rest of the Insecta.

In consideration of all these points it is clear that Anurida possesses certain characters allying it closely to the lower arthropod groups. The holoblastic cleavage and egg membranes ally it to both crustaceans and myriapods, while the structure of the ovary is most like the synthetic type Scolopendrella, but more like the chilognath myriapod than the chilopod. In spite of the possession of some generalized characters, it is evident that Anurida is a degenerate type that has been developed by a lengthening of embryonic life and a shortening of adult life. Paedogenesis, the sexual maturing of a larva, is illustrated by this process. The absorption of the embryonic yolk by the reproductive organs and the great maturity of the products even immediately after hatching both point to a tendency to shorten adult life and to omit larval development even to the extent of assuming the larval form for the adult. The decrease in the number of abdominal segments is only another step in the same direction. If the insect may be considered a larval chilognath sexually matured and bearing the three pairs of legs found in the chilognath larva, so can an Anurida be considered a very simple insect embryo matured sexually. Observations have been before advanced to establish the progressive shortening in some forms and gradual elimination of larval forms.

Anurida shows additional interesting points. By its curious habitat, chiefly under water, it has lost the need for tracheae, and, consequently, they are so far obliterated as to be absent even in the embryo; its respiration is purely cutaneous. It has been remarked that the amnion and serosa, the cellular envel-

opes of higher tracheates, are connected strictly with terrestrial forms and are one of the necessary adaptations to the exigencies of land life.   Whether or not the ancestors of Anurida ever possessed such structures and have since lost them in consequence of acquired semi-aquatic life cannot be settled, but it is interesting to speculate on the possibilities of Anurida being a simple form and still retaining a semi-aquatic mode of life and showing a few transitional characters.

Notes on Other Points of Interest.

Nervous System. — No detailed observations were made on the development of the central nervous system, but a few points of correspondence with other forms were noted.   The brain and ventral cord both arise in the same way as that described by Wheeler ('93) for Xiphidium, — by the proliferation from single ectoderm cells until rows of nerve.cells arise.   Proliferation is in the direction of the dorso-ventral axis of the embryo, and is restricted to certain places in the segments; subsequently, these primitive ganglia are united to form the ventral cord. Ultimately the six abdominal ganglia are fused to form a mass. The brain portion may be readily seen to have the three successive segments, protocerebrum, deutocerebrum, and tritocerebrum.   The optic lobes form a large part of the young protocerebrum.   Above the stomodeal invagination soon arose by proliferation from the hinder end a cord similar in structure to the ectoderm of the invagination that after reaching a considerable length remains unchanged, but a prominent character even in late embryonic life.   It is entirely missing in the young animal and nothing remains to suggest its former presence. From the method of origin it is concluded that this is a trace of a sympathetic system, this being the history of this system in other insects; but since the adult seems to lack a sympathetic system, its degeneration is to be expected.   Pauropus, that low, degenerate myriapod, also lacks a sympathetic system. These are mere notes on the general features of the nervous system, and a more complete study will be reserved for a future occasion.

Respiratory System. — Anurida, as has long been known, lacks entirely any tracheal system ; respiration is carried on wholly by means of the skin. In the embryos no invaginations were seen to represent even the rudiments of such a system. There are, however, at the bases of the legs and at different parts of the abdomen large unicellular glands that may have some relations to tracheal openings or the different glands found in myriapods and other Tracheata. This point, too, remains for further investigation.

In conclusion I wish to acknowledge the helpful oversight given to me during this investigation by the Department of Zoölogy of the University of Chicago, where the greater part of the work was done. My thanks are especially due to Dr. W. M. Wheeler, under whose direct supervision the subject was undertaken. I am also indebted to him for many valuable suggestions in the treatment of the subject and in methods and much assistance in reaching literature, and I wish here to express my grateful appreciation of the aid so freely given.

Since finishing this article, two contributions have been made to our knowledge of the development of the Apterygota, in both cases of the Thysanura. One article is by Dr. Heinrich Uzel, in the *Zoologischer Anzeiger* (Bd. XX, Nrs. 528, 529, and 535) for 1897, entitled "Beiträge zur Entwicklung der Thysanuren" (*Campodea staphylinus* Westw. and *Lepisma saccharina* L.). Another is by Dr. R. Heymons, published in the *Zeitschrift für wissenschaftliche Zoologie* for 1897, on the subject of "Entwicklungsgeschichtliche Untersuchungen an Lepisma saccharina, L." There are many points of interest between the observations made by these authors on the Thysanura and those given above for Anurida; one or two in particular will be briefly mentioned.

Both authors describe the cleavage as distinctly superficial in Lepisma, and Uzel observes the same to be true in the spherical egg of Campodea. This is an interesting point in

consideration of the sizes and shapes of the eggs in the three forms. In Lepisma the egg is a regular oval, and about 1 mm. in its longest diameter; in Campodea the egg is spherical, and has a diameter of about 0.4 mm., while the egg of Anurida has also a spherical form, but is only about 0.27 mm. in diameter. This increase in size is enough to explain the loss of holoblastic cleavage in the larger forms, considering its imperfect preservation in·the small anuridan egg.

The process of germ band formation as described by Uzel for Campodea agrees very closely with the corresponding one in Anurida. A "dorsal organ" is described as having a position comparable to that held by the precephalic organ in Anurida. There are in Campodea no embryonic membranes corresponding to the amnion and serosa of the pterygote insect. In Lepisma these structures appear, but the amniotic sac remains open for a short distance. In many ways Campodea suggests to Uzel the myriapod type of development.

In the more complete consideration of Lepisma by Heymons there are several special points of interest. He finds rudimentary appendages upon the tritocerebral segment, which eventually disappear in early embryonic life. In discussing the origin of the mesenteron, several observations agree closely with those made on Anurida. The appearance of this part of the alimentary tract is very much delayed. Not until after hatching is it definitely formed. Unfortunately, certain critical stages were not found; but the author saw in late embryonic stages small groups of cells taking up a peripheral position on the yolk. These groups increase by rapid division originating from what the author considers yoke cells that have been functioning throughout embryonic life in the assimilation of yolk. Hence he says the mesenteron is truly entodermic in origin. There is only one principal point of difference between this view and the one given for Anurida. In the latter case, it is clear that the mesenteron arises from cells originating at the same time as the yolk cells, but remaining latent through the early embryonic stages; the yoke cells themselves degenerate at the close of embryonic life. Possibly there is some such latent source in Lepisma that may have escaped observation.

The origin of the germ cells is another question of extreme interest. It was only after prolonged study that the interpretations of the facts observed in Anurida, as given above, were formulated. One point observed in Lepisma corroborates in part the conditions described in Anurida. It is clear that, as in Blatta, the germ cells are subject to great changes of position. According to Heymons they have an ectodermal origin, and appear early in embryonic life. The formation of egg tubes and their connection with each other are all steps accomplished by the process of migration. There is clearly nothing metameric in their origin, and any such arrangement must be secondary. At present nothing further can be said on the diverse origins of the germ cells, — from the ectodermic in one case and mesoderm in the other.

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REFERENCE LETTERS.

$a_1.-a_6.$	abdominal segments	$m.g.c.$	migrating germ cells.
$an.$	anus.	$mp.$	mouth-parts.
$a.o.$	abortive ovum.	$m.pr.$	male pronucleus.
$at.$	antenna.	$mx_1.$ $mx_2.$	maxillae.
$b.c.$	blood corpuscle.		
$bl.$	blastomeres.	$n.$	nucleus.
$c_1.$ $c_2.$	crenated membranes.	$n.c.$	nutritive cells.
		$ncl.$	nucleolus.
$c.b.$	central blastomeres.	$nr.$	nervous system.
$c.el.$	cephalic elongation.	$o.$	ovum.
$c.g.$	cavity of mid-gut.	$o.d.$	oblique division.
$ch.$	chromatin.	$ov.$	ovary.
$cl.$	collophore.	$p.$	preparatory membrane.
$c.p.$	central mass of protoplasm.	$p.b_1.$ $p.b_2.$	polar bodies.
$dc.$	deutocerebrum.		
$e.$	eye.	$p.c.$	protocerebrum.
$ec.$	ectoderm.	$pc.o.$	precephalic organ.
$e.m.$	egg membrane.	$pd.$	proctodaeum.
$en.$	entoderm.	$p.i.$	protoplasmic island.
$f.$	follicle.	$p.n.$	protoplasmic network.
$f.g.$	fat globules.	$r.$	radial protoplasm.
$f.pr.$	female pronucleus.	$r.o.$	opening for reproductive organ.
$g.c.$	germ cell.	$s.$	sperm cells.
$g.e.$	germinal epithelium.	$s.g.c.$	stationary germ cells.
$g.v.$	germinal vesicle.	$sp.me.$	splanchnic mesoderm.
$i.c.$	intercalary segment.	$st.$	stomodaeum.
$int.$	intestine.	$t_1.$ $t_2.$ $t_3.$	thoracic segments.
$k.$	knob.		
$l.m.$	longitudinal muscle.		
$lb.$	labrum.	$t.a.$	thoracic appendages.
$m.$	membrane.	$tc.$	tritocerebrum.
$mc.$	muscles.	$y.$	yolk.
$md.$	mandibles.	$y.c.$	yolk cells.
$me.$	mesoderm.	$y.m.$	yolk mass.
$me.s.$	mesoblastic somite.	$y.n.$	yolk nucleus.
$m.f.$	mouth fold.	$v.$	vitelline membrane.
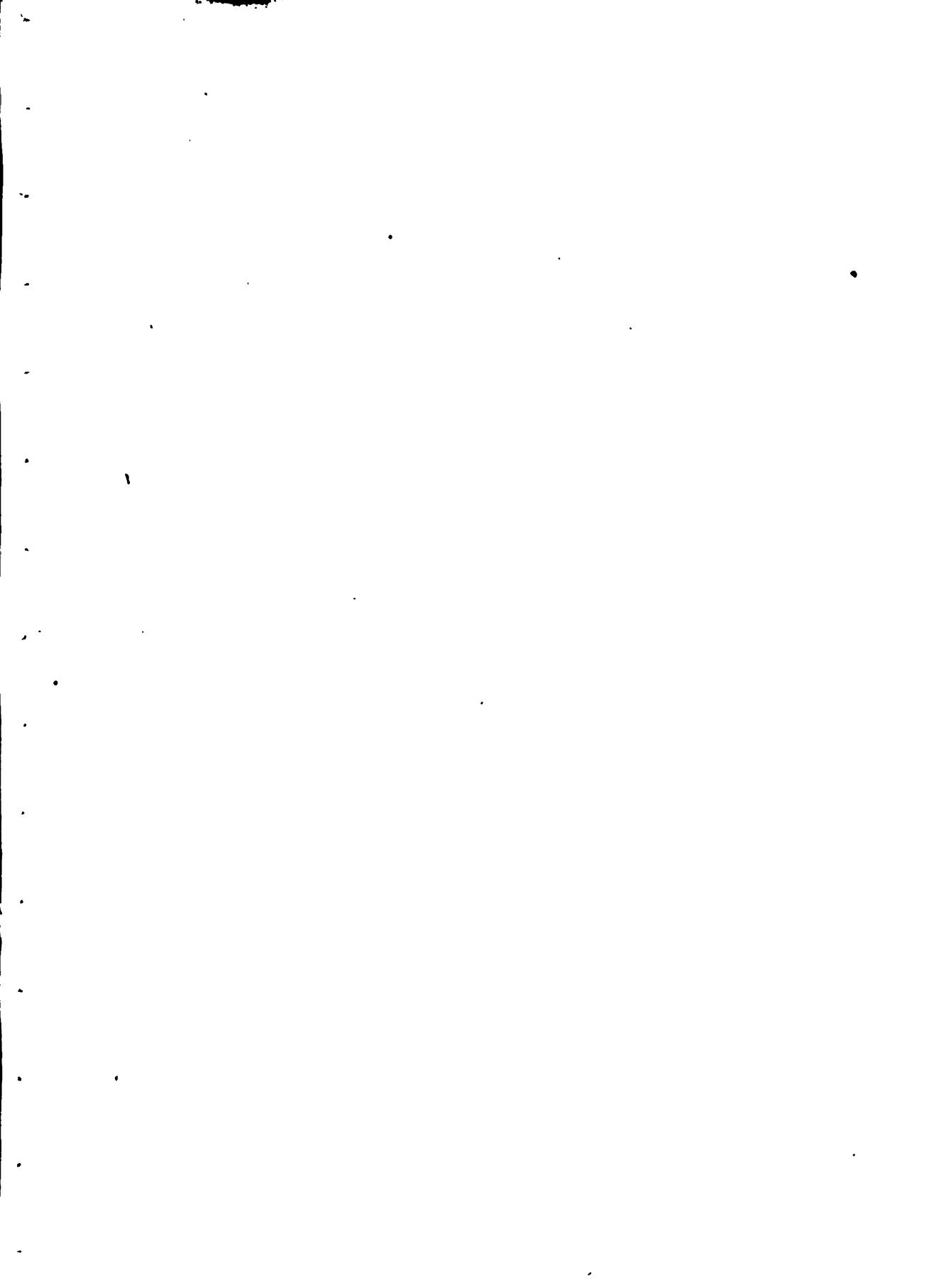
EXPLANATION OF PLATE XX.

NOTE. *Unless otherwise stated drawings are made with Zeiss lenses and the ordinary tube length. Objective $\frac{1}{12}$ is an oil immersion.*

Anurida maritima Guen., Figs. 1–13; stained in lithium carmine and Lyons blue, Fig. 14. *Tomoceras sp. ?*

FIG. 1. Longisection of adult ovary in early summer. *c.el.*, cephalic elongation attached to the fat body; *o.*, young ova; *n.c.*, nutritive cells associated with ova; *g.e.*, germinal epithelium; *ov.*, ovarian wall. Obj. 4, oc. 4.

FIG. 2. Group of germ cells from near the germinal epithelium, showing the characteristic way of association. Letters as in Fig. 1. Obj. $\frac{1}{12}$, oc. 6.

FIG. 3. Ovum at later stage, when the cytoplasm is increased. Letters as above. Obj. $\frac{1}{12}$, oc. 6.

FIG. 4. Section of ovary in younger stage than represented in Fig. 1, yolk not yet appearing. *p.n.*, cytoplasm of ovum, highly vesicular; *y.n.*, yolk nucleus of nutritive cells; *f.*, beginning of follicle. Obj. $\frac{1}{12}$, oc. 4.

FIGS. 5 and 6. Transections through cephalic elongation, showing non-germinal character of cells. Obj. $\frac{1}{12}$, oc. 4.

FIG. 7. Longisection through ovary in region of beginning of cephalic elongation. Letters as above. Obj. $\frac{1}{12}$, oc. 2.

FIG. 8. Young ovum and nutritive cells. Letters as above. Obj. $\frac{1}{12}$, oc. 6.

FIG. 9. Enlarged view of one of the small masses shown in Fig. 1. Letters as in 8. Obj. $\frac{1}{12}$, oc. 6.

FIG. 10. Enlarged view of an egg mass from ovary shown in Fig. 1. *ch.*, chromatin; *ncl.*, nucleolus; *n.*, nucleus of nutritive cells and ovary; *a.o.*, abortive ovum; *y.*, yolk appearing in ovum. The nucleus is already invisible. Obj. $\frac{1}{12}$, oc. 4.

FIG. 11. Late stage of ovarian development, showing parts of two eggs. Letters as above. Obj. $\frac{1}{12}$, oc. 2.

FIG. 12. Final stage in ovarian development. *e.m.*, egg membrane. Obj. $\frac{1}{12}$, oc. 2.

FIG. 13. Enlarged view of abortive ovum and nutritive cell. *ncl.*, nucleolus; rest of letters as in Fig. 10. Obj. $\frac{1}{12}$, oc. 4.

FIG. 14. Ovum from ovary of Thysanuran (*Tomoceras*), showing the beginning of yolk formation and the preservation of the group of chromosomes. Letters as before. Obj. $\frac{1}{12}$, oc. 3.
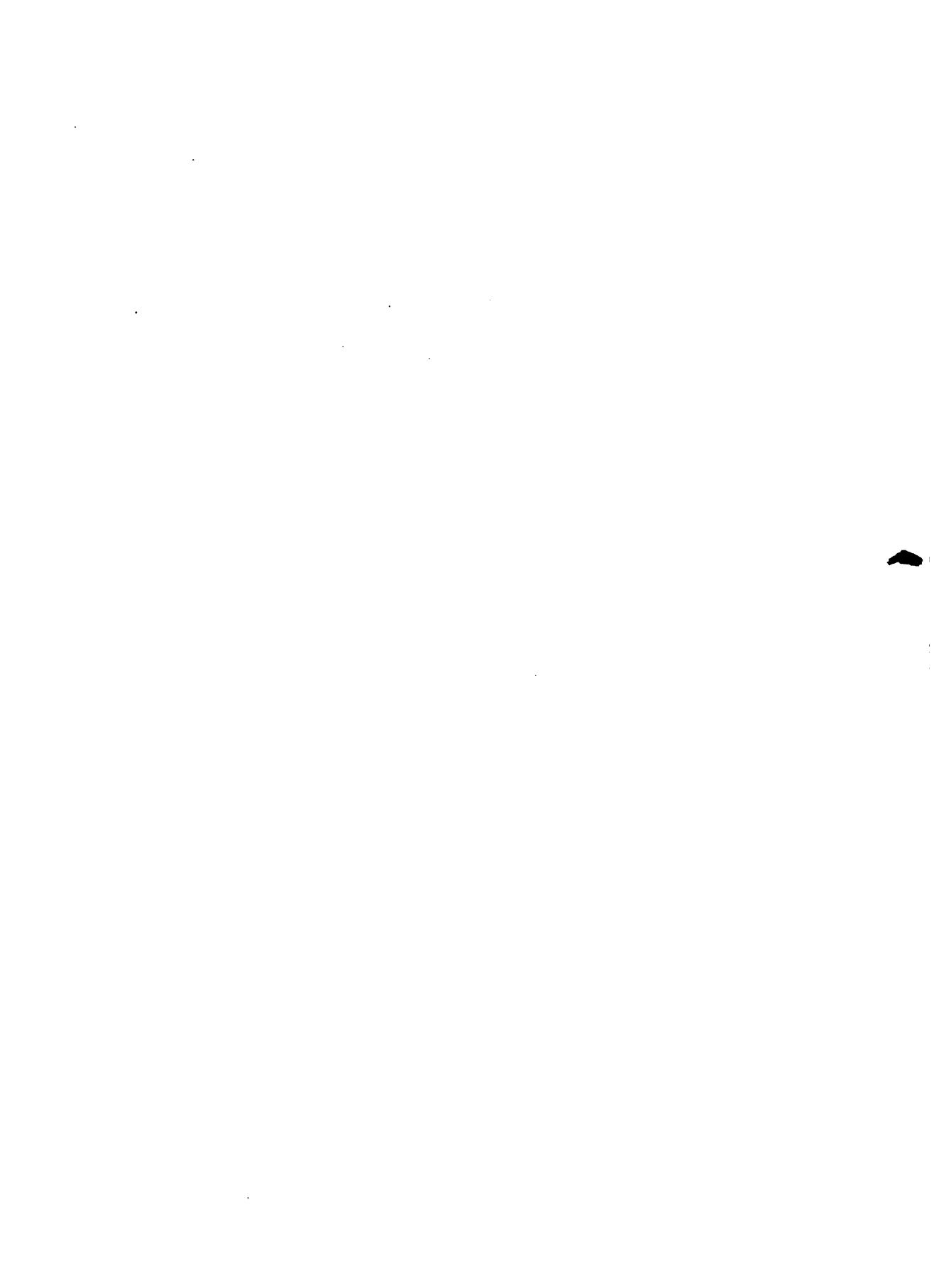
EXPLANATION OF PLATE XXI.

Cleavage.

FIG. 15.   External view of unsegmented eggs, showing grouping.   Obj. 4, oc. 2.

FIGS. 16–24 are surface views of cleavage stages, showing the 2, 4, 8, 16, 32, and coarse morula stages until total cleavage ceases.   Drawn from unstained eggs.   Obj. 16, oc. 6.

FIG. 25.   Protoplasmic mass at the surface of egg.   *f.pr.*, female pronucleus returning to centre; *p.b.*, polar bodies.   Borax carmine.   Obj. $\frac{1}{12}$, oc. 6.

FIG. 26.   Showing division of first polar body.   Letters as in Fig. 25.   Borax carmine.   Obj. $\frac{1}{12}$, oc. 6.

FIG. 27.   Part of protoplasm from the centre of the unsegmented egg.   *f.pr.*, female pronucleus; *m.pr.*, male pronucleus.   Borax carmine.   Obj. $\frac{1}{12}$, oc. 6.

FIG. 28.   First cleavage spindle.   Iron haematoxylin and Orange G.   Obj. $\frac{1}{12}$, oc. 2.

FIG. 29.   Reconstruction of nucleus after division into the 2-cell stage.   Letters as before.   Iron haematoxylin and Orange G.   Obj. 4, oc. 4.
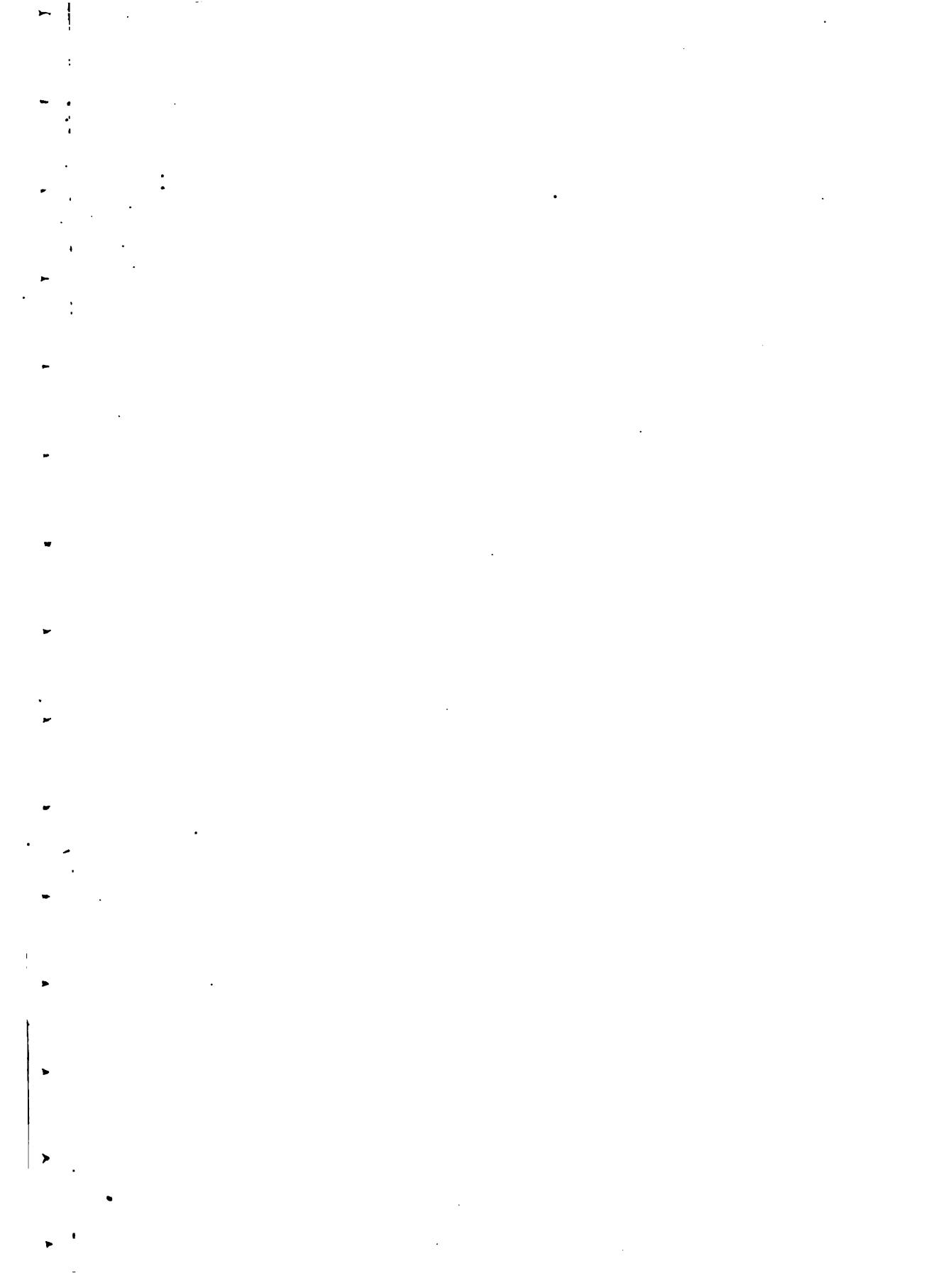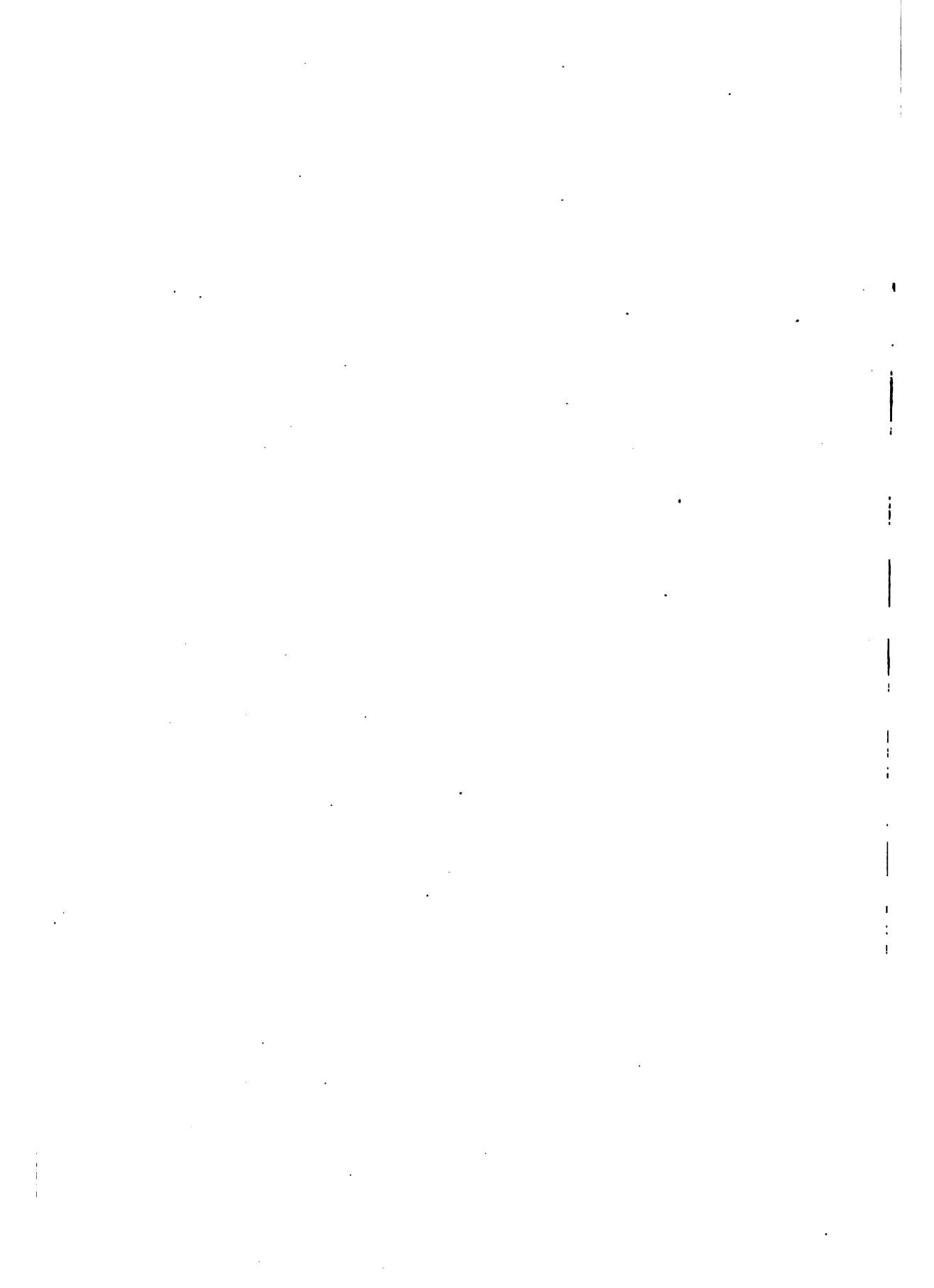
EXPLANATION OF PLATE XXII.

Blastoderm Formation.

FIG. 30.   Section through unsegmented egg.   *p.i.*, protoplasmic island in which female pronucleus is present; *c.p.*, central mass of protoplasm; *r.*, radial protoplasmic strands; other letters as before.   Borax carmine.   Obj. 4, oc. 6.

FIG. 31.   Section through line Z–Z in Fig. 21.   *bl.*, blastomere; *e.m.*, egg membrane; *v.*, vitelline membrane.   Iron haematoxylin and Orange G.   Obj. 8, oc. 6, tube length $15\frac{1}{2}$.

FIG. 32.   32-cell stage.   *c.b.*, central blastomeres.   Erlich's haematoxylin. Obj. 4, oc. 6.

FIG. 33.   Part of egg after holoblastic cleavage has ceased.   *bl.*, blastomeres from which nuclei surrounded by protoplasm are migrating.   Borax carmine. Obj. 4, oc. 6.

FIG. 34.   Early blastoderm.   *ec.*, ectoderm ; *me.*, mesoderm ; *y.m.*, yolk masses showing earlier position of blastomeres ; *o.d.*, oblique division of ectoderm cells. Erlich's haematoxylin.   Obj. 6, oc. 6.

FIG. 35.   Blastoderm formation completed.   *pc.o.*, beginning of the precephalic organ ; *en.*, entoderm cells ; *y.c.*, yolk cells ; the rest as in Fig. 34.   Borax carmine.   Obj. 8, oc. 6.

FIG. 36.   Later stage of blastoderm.   Letters as in Fig. 35.   Borax carmine. Obj. 8, oc. 6.

FIG. 37.   Still later stage.   Letters as in Fig. 35.   Borax carmine.   Obj. 4, oc. 1.

FIG. 38.   Precephalic organ at its period of greatest development, blastoderm crenated.   *c₁.*, first crenated membrane; remaining letters as before.   Borax carmine.   Obj. 4, oc. 1.

FIG. 39.   Precephalic organ elongated.   *c₂.*, second crenated membrane; *k.*, knob where elongation is attached to crenated membrane number 2 ; remaining letters as above.   Borax carmine.   Obj. 4, oc. 1.

FIG. 40.   Enlarged view of section of membranes and ectoderm of ovum.   *p.*, preparatory membrane; other letters as before.   Borax carmine.   Obj. $\frac{1}{12}$, oc. 6.

FIG. 41.   Entoderm cells showing vesicular protoplasm.   Borax carmine. Obj. $\frac{1}{12}$, oc. 4.
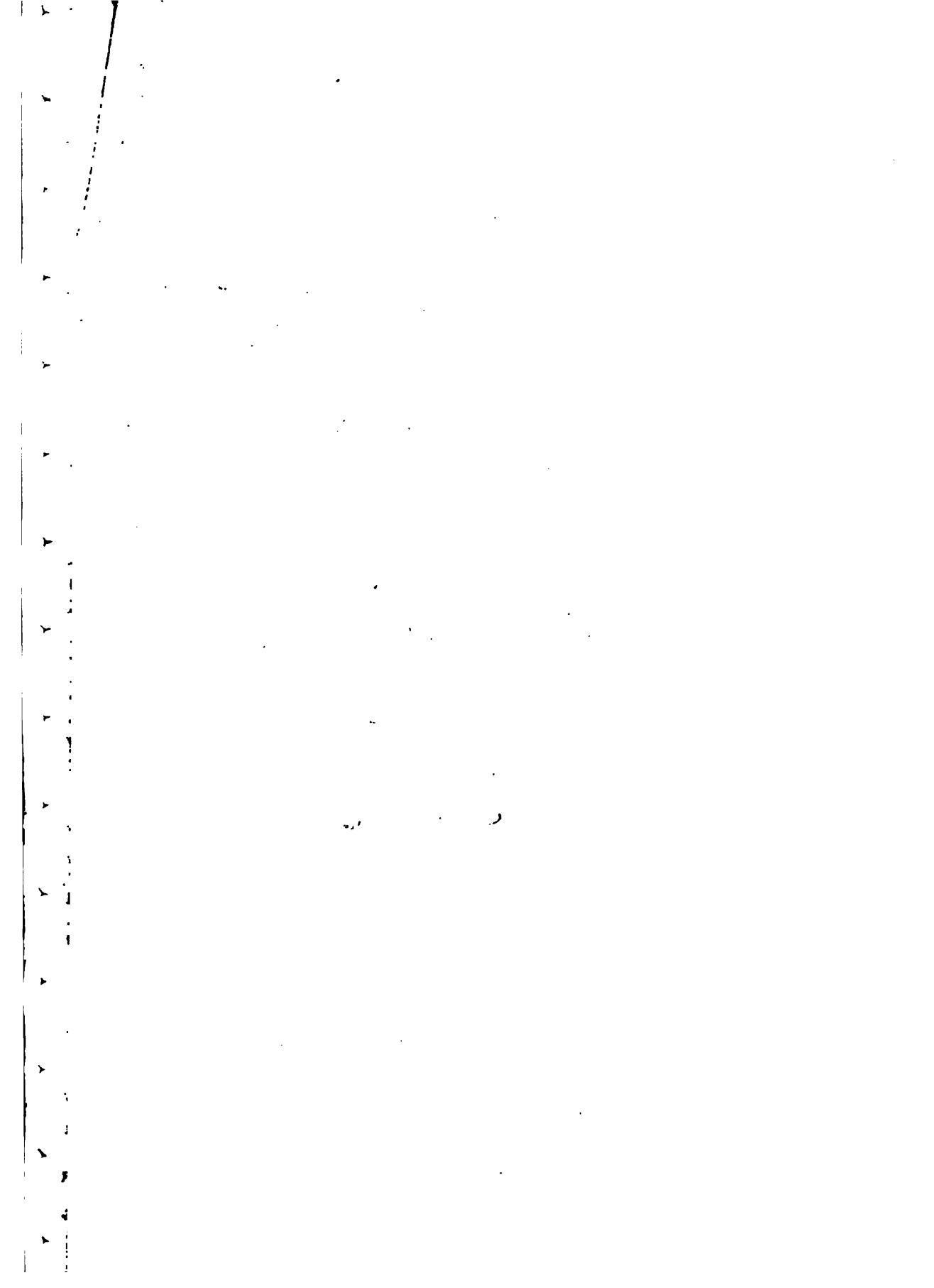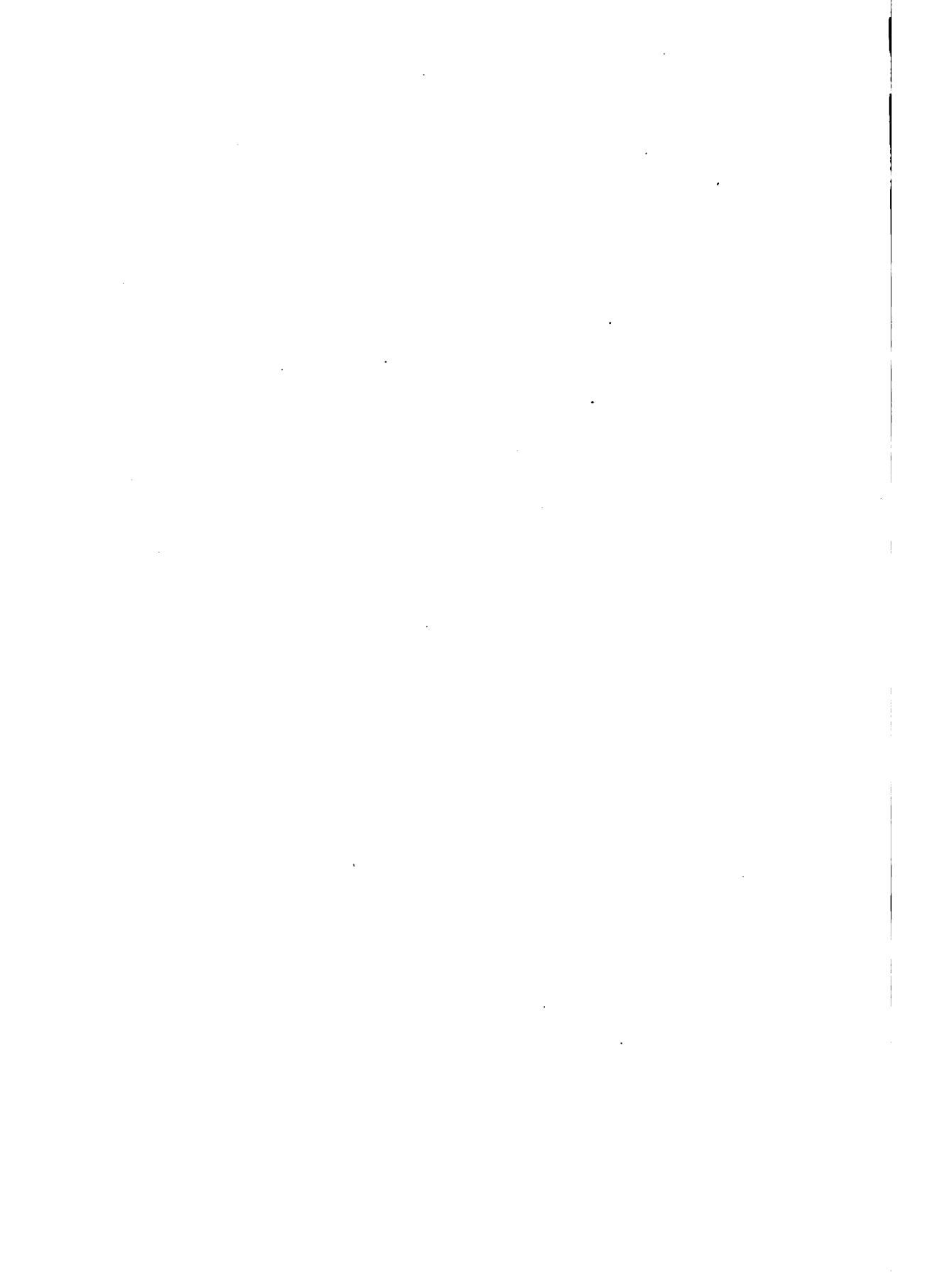
EXPLANATION OF PLATE XXIII.

Surface Views of Embryos. Erlich's Haematoxylin.

FIG. 40. Ventral view of early embryo. The embryo is rolled over and represented as laid out flat. *at.,* antenna, *i.c.,* intercalary appendage (2d antenna) ; *md.,* mandibles; mx_1., mx_2., maxillae ; t_1., 1st thoracic legs; *pc.o.,* precephalic organ. Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 41. t_2., t_3., 2d and 3d thoracic legs; a_1., 1st abdominal appendage ; other letters as above. Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 42. *lb.,* labrum; a_1.–a_5., abdominal segments and beginning of appendages ; *pd.,* proctodaeum; remaining letters as in Fig. 41. Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 43. Face view of later embryo, shown in side view in 43*a.* Flexure just beginning. *m.f.,* mouth fold; *m.p.,* mouth-parts ; *t.a.,* thoracic appendages; *cl.,* collophore ; a_2.–a_4., appendages on abdomen. Obj. 4, oc. 6, tube length $15\frac{1}{2}$.

FIG. 43*a.* Outline side view of embryo represented in Fig. 43, showing beginning of flexure. Obj. 16, oc. 4, tube length $15\frac{1}{2}$.

FIG. 44. *pc.o.,* precephalic organ elongating ; *m.,* last membrane shed ; other letters as above. Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 45. Flexure almost complete. *e.,* eyes ; remaining letters as before. a_4. is larger than any of other abdominal appendages excepting the collophore. Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 46. Head of embryo showing the beginning of the mouth fold, *m.f.* Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 47. Enlarged head of embryo of same stage as Fig. 42. *pc.,* procerebrum ; *dc.,* deutocerebrum ; *tc.,* tritocerebrum bearing *i.c.,* the intercalary appendage. Obj. 8, oc. 4, tube length $15\frac{1}{2}$.

FIG. 48. Newly hatched young *Anurida maritima.* Letters as before. Reichert, obj. 3, oc. 1.
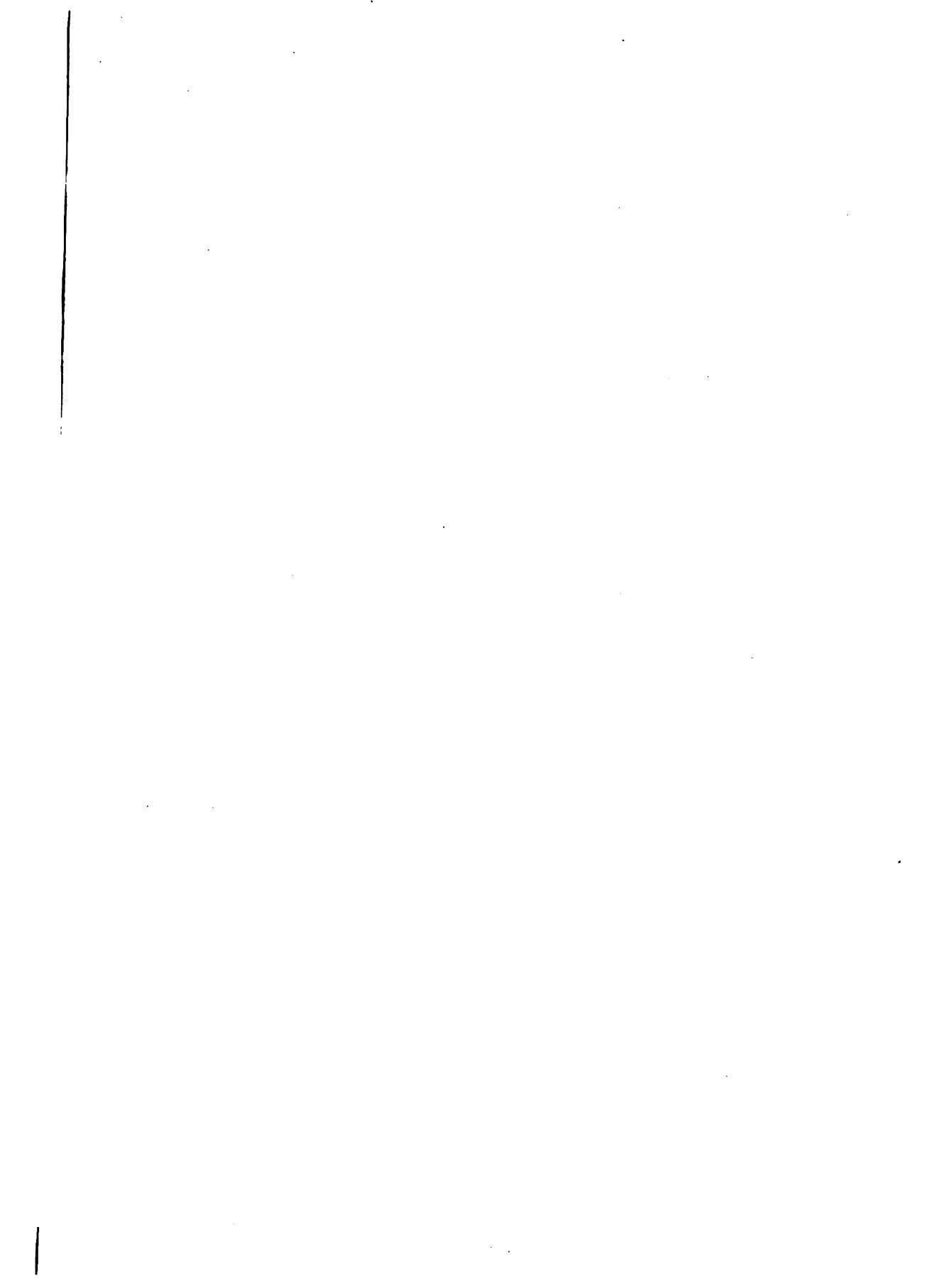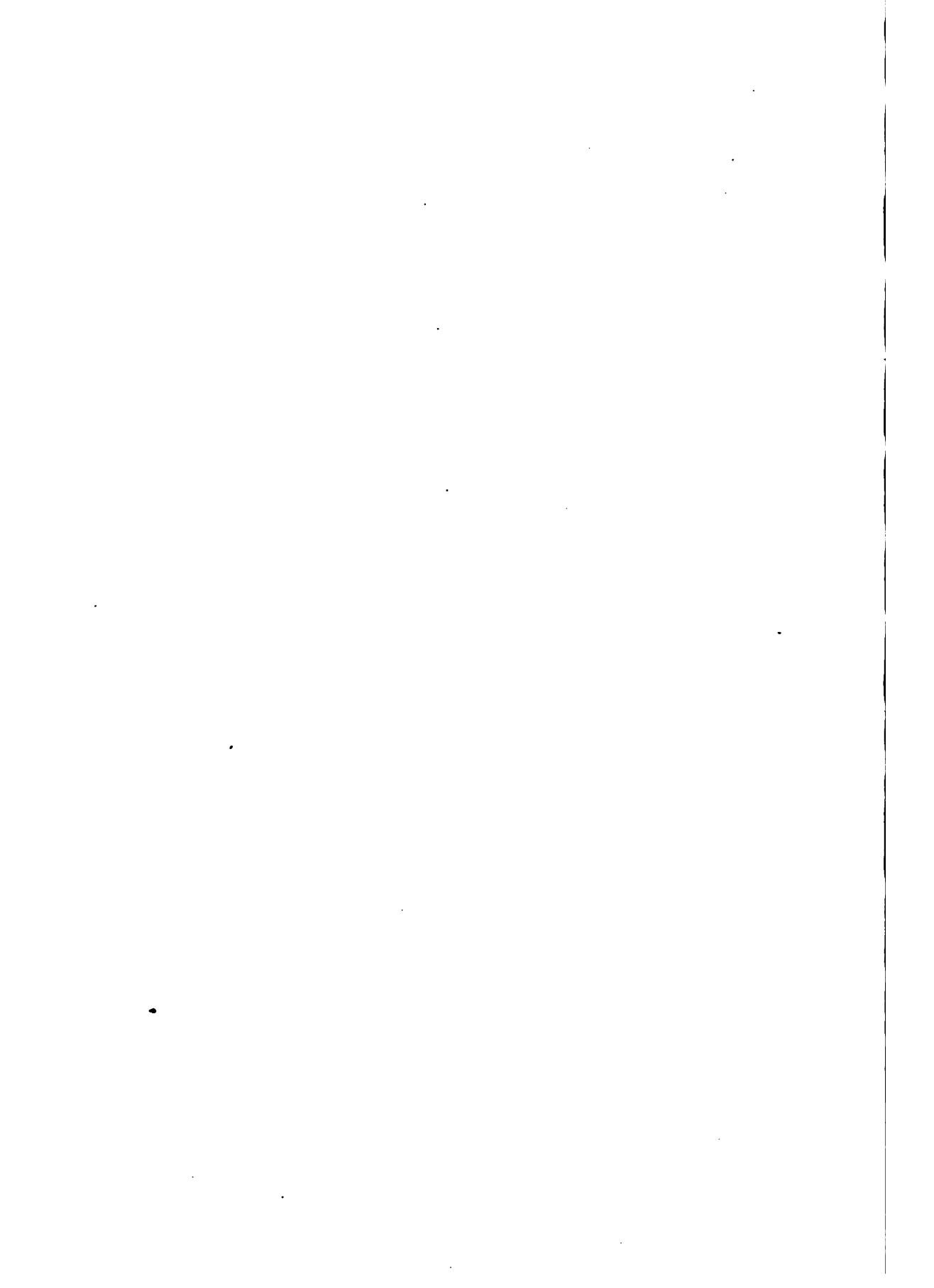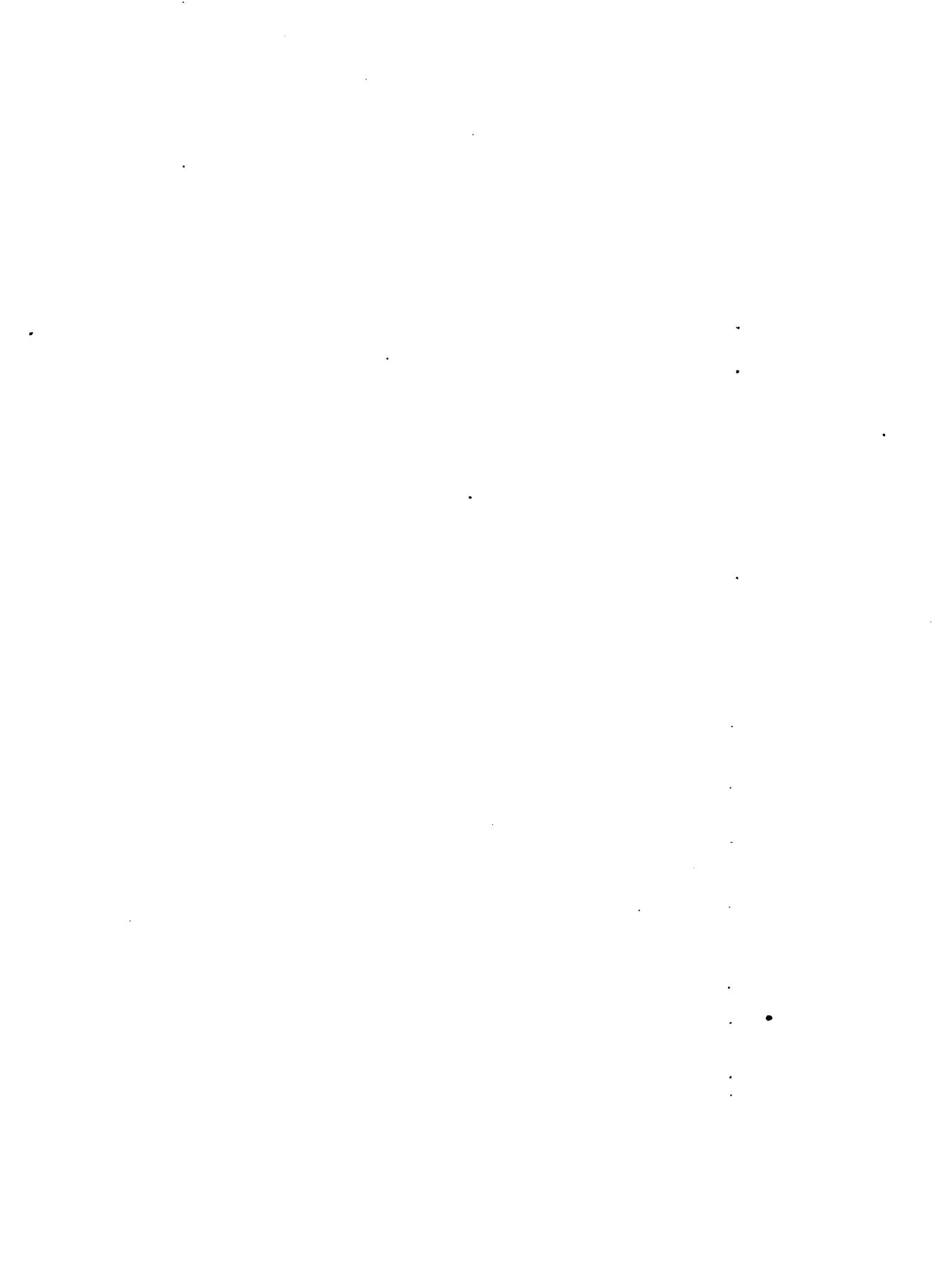
EXPLANATION OF PLATE XXIV.

Development of the Reproductive Cells.

FIG. 49. Transection showing early mesoderm formation. Letters as before. Borax carmine. Obj. $\frac{1}{15}$, oc. 2, tube length 15½.

FIG. 50. Transection through somite on one side of body in embryo of age shown in Fig. 44, 2d abd. segment. *g.c.*, germ cells. Delafield's haematoxylin. Obj. $\frac{1}{15}$, oc. 6, tube length 15½.

FIG. 51. A similar section of same age as in Fig. 50. *me.s.*, mesoblastic somite, cavity distinct. Borax carmine. Obj. $\frac{1}{15}$, oc. 6, tube length 15½.

FIG. 52. Longitudinal section through under part of abdomen of later embryo. *mc.*, beginning of muscles; other letters as before. Delafield's haematoxylin. Obj. $\frac{1}{15}$, oc. 4, tube length 15½.

FIG. 53. Longisection through abdomen of stage shown in Fig. 45. *sp.me.*, splanchnic layer of mesoderm; *bl.c.*, blood corpuscles; other letters as above. Delafield's hematoxylin. Obj. $\frac{1}{15}$, oc. 4, tube length 15½.

FIG. 54. Longisection through hinder part of abdomen of embryo in corresponding stage (slightly oblique). *an.*, anus; rest of letters as before. Germ cells are migrating into the yolk. Delafield's haematoxylin. Obj. $\frac{1}{15}$, oc. 4, tube length 15½.

FIG. 55. Longisection through embryo. *me.*, two layers of mesoderm, splanchnic and somatic. Delafield's haematoxylin. Obj. $\frac{1}{15}$, oc. 6, tube length 15½.

FIG. 56. Similar section to Fig. 55. *s.g.c.*, stationary germ cells; *m.g.c.*, migrating germ cells. Delafield's haematoxylin. Obj. $\frac{1}{15}$, oc. 6, tube length 15½.

FIG. 57. Section showing migrated germ cells and scattered degenerating yolk nuclei, *y.c.* Obj. $\frac{1}{15}$, oc. 4, tube length 15½.

EXPLANATION OF PLATE XXV.

FIG. 58.   Longisection through abdomen of last stage of embryo. *en.*, ento-derm.   Other letters as before.   Borax carmine and Orange G.   Obj. $\frac{1}{12}$, oc. 4-tube length 15½.

FIGS. 59, 60, 62.   Cross-sections of ovaries of just-hatched Anurida. *y.*, em-bryonic yolk ; rest of letters as before.   Borax carmine and Orange G.   Obj. $\frac{1}{12}$, oc. 4, tube length 15½.

FIG. 61.   Cross-section of small animal taken early in the summer.   Erlich's haematoxylin.   Obj. $\frac{1}{12}$, oc. 4, tube length 15½.

FIG. 63.   Blood corpuscles from just-hatched animal containing yolk.   Borax carmine.   Obj. $\frac{1}{12}$, oc. 6, tube length 15½.

FIG. 64.   Slightly oblique longisection of just-hatched male. *l.*, longitudinal muscles ; *f.g.*, fat globules ; *int.*, intestine ; *r.o.*, opening of reproductive organs; *n.c.*, nerve cord ; other letters as before.   Erlich's haematoxylin.   Obj. 8, oc. 4, tube length 15½.

FIG. 65.   Longisection through mid-gut of just-hatched male. *en.*, entoderm ; *y.*, yolk ; *c.g.*, cavity of gut; *s.*, sperm cells; *g.e.*, germinal epithelium.   Borax carmine and Orange G.   Reichert, obj. $\frac{1}{12}$, oc. 1.

FIG. 66.   Frontal view of Petromyzon from a drawing by Dr. W. M. Wheeler. This shows the association of yolk with germinal cells.
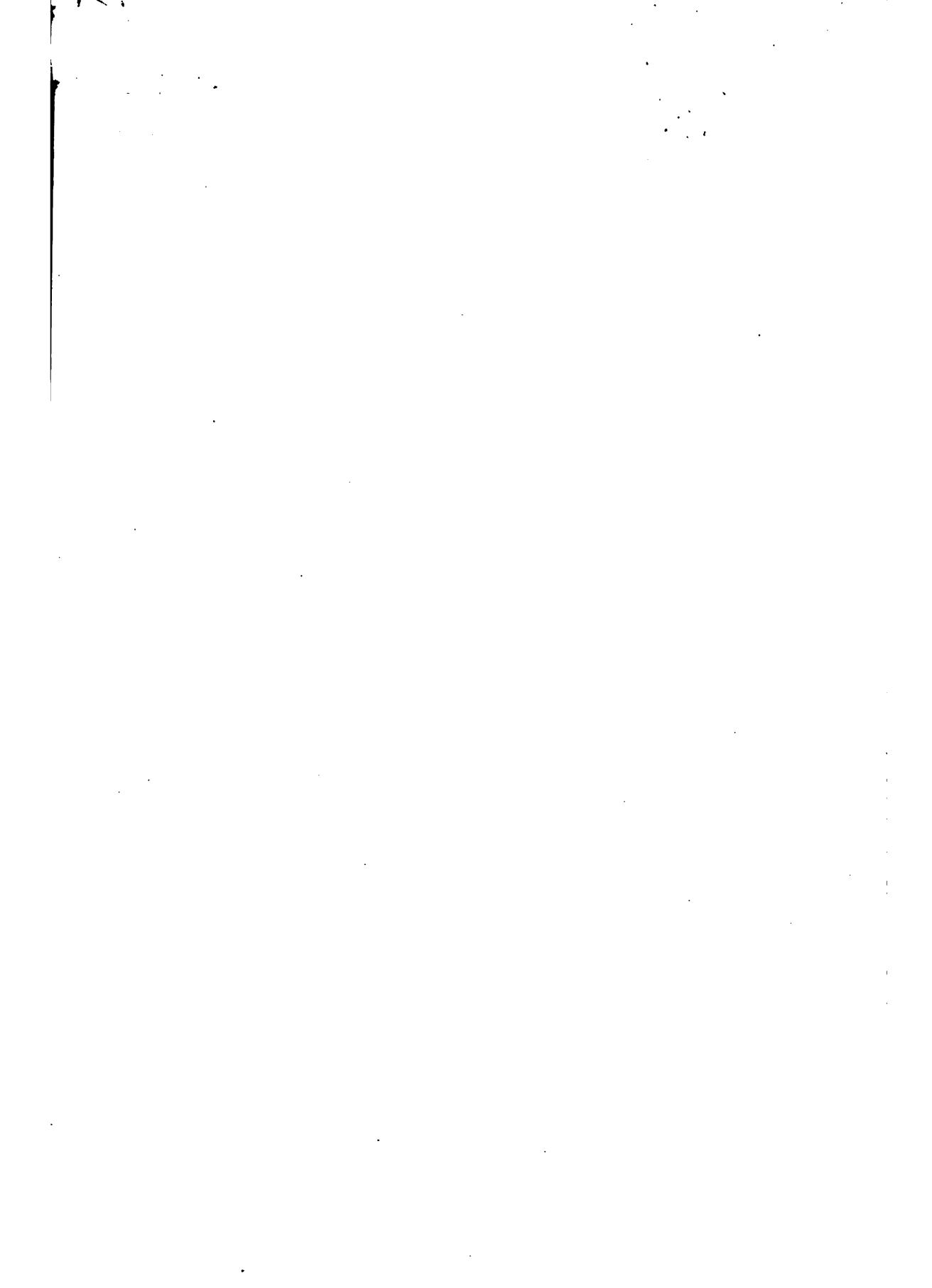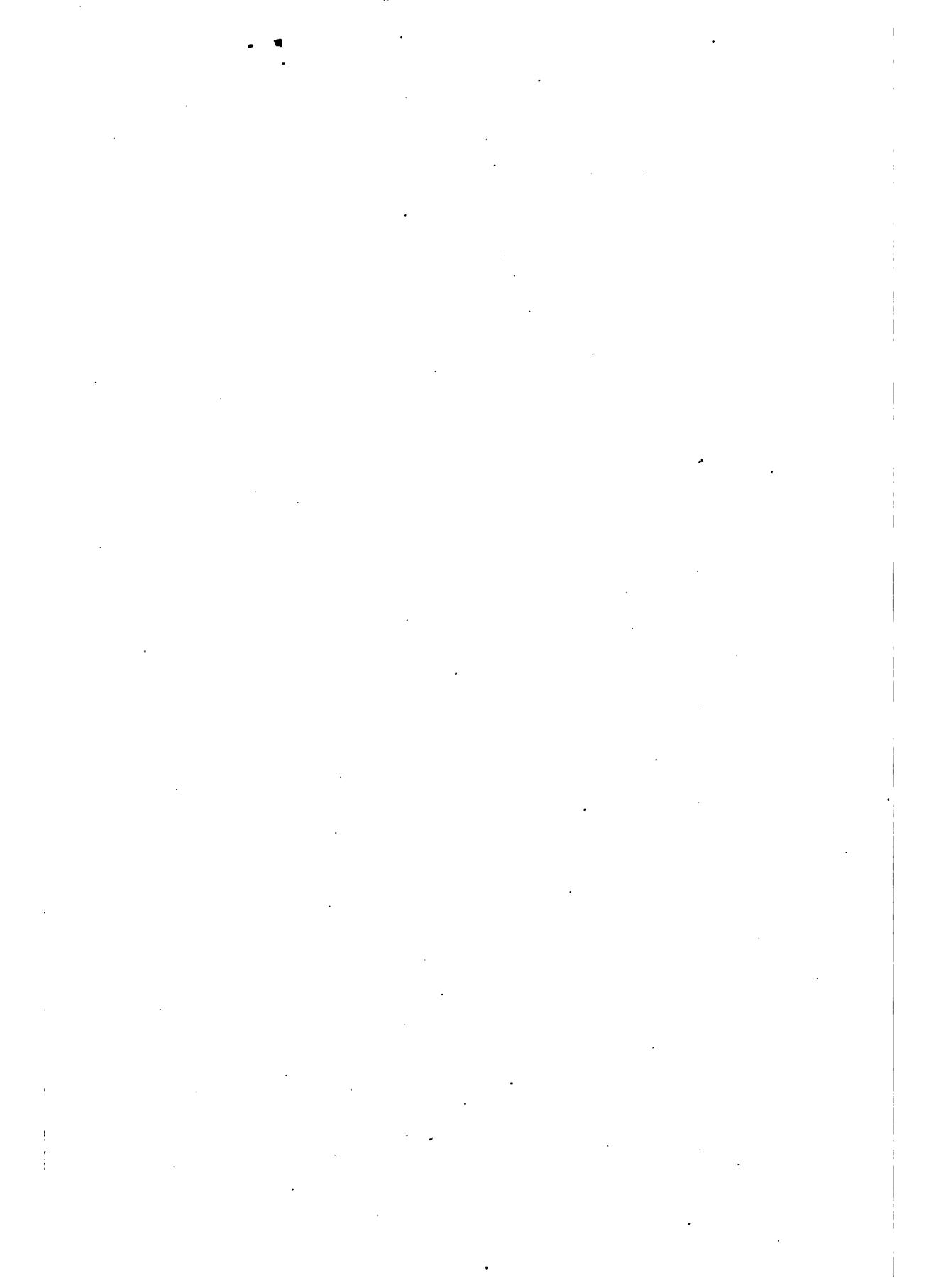
The University of Chicago
FOUNDED BY JOHN D. ROCKEFELLER

THE EMBRYOLOGY AND OÖGENESIS OF ANURIDA MARITIMA

(GUÉR)

A DISSERTATION

SUBMITTED TO THE FACULTIES OF THE GRADUATE SCHOOLS OF ARTS,
LITERATURE, AND SCIENCE, IN CANDIDACY FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

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